Supervisors: Prof. dr. ir. Pascal Boeckx
Isotope Bioscience Laboratory (ISOFYS),
Department of Applied Analytical and Physical Chemistry
Faculty of Bioscience Engineering
Ghent University, Gent, Belgium

Prof. dr. ir. Jan Diels
Department of Earth and Environmental Sciences
KU Leuven - University of Leuven, Belgium

Dean: Prof. dr. ir. Guido Van Huylenbroeck
Rector: Prof. dr. Anne De Paepe

Amsalu Nebiyu Woldekirstos

# Role of faba bean (*Vicia faba* L.) for intensification of cereal-based cropping systems in the humid highlands of Ethiopia

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD)
in Applied Biological Sciences

Dutch translation of the title:

Rol van fababonen (*Vicia faba* L.) voor intensivering van teeltsystemen op basis van granen in de vochtige hooglanden van Ethiopië

Illustration on the front cover:

The faba bean (*Vicia faba* L.) crop at early flowering in the highlands of Dedo, Southwestern Ethiopia

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# Summary

In most cereal cropping systems of the African tropical highlands, phosphorus (P) and nitrogen (N) fertilizer application is limited. Maintaining sufficient available P and N for cereal production is a major challenge. In this context, biological nitrogen fixation (BNF), P acquisition (PAE) and utilization efficiency (PUE) are critical factors for sustainable agronomic intensification of tropical highland agriculture. The use of P efficient and $N_2$-fixing grain legumes is a prerequisite for sustainable intensification of low input cereal-based agro-ecosystems.

The study was carried out on farmer's fields in highland cropping systems of southwest Ethiopia, which are characterized by a subsistence-oriented mixed crop-livestock farming system wherein cereal-based cropping systems dominate. A series of experiments were undertaken to determine the agronomic performance in terms of grain and biomass yield, BNF, PUE and N balance of faba bean grain legumes. Subsequently, the effect of crop residues of selected faba bean varieties on agronomic performance of wheat was investigated. Finally a follow up experiment has been performed to determine whether climate, edaphic or variety conditions control agronomic performance, BNF and PUE of faba beans.

Quantifying BNF by legumes through $\delta^{15}N$ natural abundance measurements requires correct determination of a so-called *B-value* (i.e. $\delta^{15}N$ value of a legume when completely dependent on $N_2$ fixation for growth). We experimentally determined variety specific *B-values* for six faba bean varieties and quantified to what extent variety has an effect on *B-values* and hence BNF and N balances. It was found that whole plant seed N corrected *B-values* were significantly different between varieties and varied between +0.5 ± 0.4 to -1.9 ± 1.4‰ suggesting a variable isotope fractionation during $N_2$ fixation. If the whole plant seed N corrected *B-values* were assessed against other approaches then it was noticed that BNF estimates could be biased by 34% (15% over- to 19% underestimation) when not accounting for varietal differences and seed-N.

Data on agronomic performance, BNF and PUE of the same six faba bean varieties were determined. There was a varietal difference ($P < 0.05$) in grain (3.7 ± 0.3 to 5.5 ± 0.6 t ha$^{-1}$) and total biomass yield (7.5 ± 0.8 to 10.8 ± 0.6 t ha$^{-1}$) of the faba bean varieties. Significant differences between the varieties were also demonstrated for BNF (225 to 338 kg N ha$^{-1}$). Varieties showed significant variations in PUE, but P application had no significant effect on PUE. Variety *Moti* demonstrated highest PUE of 272 kg grain kg$^{-1}$ P, which was 1.6-fold higher than the lowest PUE. This experiment demonstrated that variations in grain and biomass yield of faba beans were largely due to differences in PUE.

In a next step we investigated to which extent high BNF and P efficient faba bean varieties lead to positive agronomic effects on wheat grown in rotation. Here, a simple input-output model was used to calculate the N balance and two possible residue management scenarios were examined. Scenario-I (assuming that all aboveground biomass is exported from the field) gave a negative net N balance (kg N ha$^{-1}$) in the range of -78.4 ± 6.1 (variety *Degaga*) to -2.8 ± 8.7 (variety *Moti*) with significant differences between varieties. Scenario-II (assuming that all the above ground biomass except grains and empty pods is returned to the soil) showed that all balances were significantly improved and all faba bean varieties were positively contributing N to the cereal cropping system in the range 58.7 ± 13.4 (variety *Degaga*) to 174.8 ± 13.7 (variety *Moti*) kg N ha$^{-1}$, which is equivalent to 126 - 380 kg N ha$^{-1}$ in the form of urea (46% N). Biomass and grain yield of wheat grown after faba bean residue incorporation improved significantly by 112 and 82%, respectively, compared to the yield of wheat after wheat. P application to the preceding faba bean varieties significantly improved the total biomass and grain yield of the succeeding wheat with significant linear relationship between P application to the previous faba beans and wheat biomass and grain yield.

Finally, a mesocosm study was undertaken on farmers' fields at two different altitudes, which are distinct in climate and soil type, namely at *Dimtu* (lower altitude; 1790 m; average yearly rainfall is 1300 ± 404 mm) and *Dedo* (higher altitude; 2160 m; average yearly rainfall is 1874 ± 77 mm) aiming at assessing whether climate, soil or variety controls agronomic performance, BNF, PUE, rhizosphere phosphatase activity and the diversity of bacteria associated with faba bean root nodules. To assess the soil effect at each of the two locations, we mutually transplanted soil between the two locations (Dimtu and Dedo sites). Grain and biomass yield and nodulation characteristics of faba beans were determined more by location (climate) than soil or variety effect. $N_2$ fixation was higher for *Dedo* (1.2 ± 0.1 g N plant$^{-1}$) compared to *Dimtu* (0.4 ± 0.0 g N plant$^{-1}$), and variety *Moti* showed a higher $N_2$ fixation (0.9 ± 0.2 g N plant$^{-1}$) compared to *Obse* (0.6 ± 0.1 g N plant$^{-1}$). Further, highest PAE by faba beans was achieved for *Moti* in *Dedo* soil at the *Dedo* location (99.0 ± 6.0 mg P plant$^{-1}$) and the lowest was for *Obse* in *Dimtu* soil at *Dimtu* (25.0 ±3.6 mg P plant$^{-1}$), but the PUE was not significantly different ($P > 0.05$). Soil type was more important in determining the rhizosphere phosphatases activity than location or variety effects. Based on 16S rRNA gene sequencing, five different genera (*Rhizobium*, *Bacillus*, *Arthrobacter*, *Staphyllococcus* and *Leifsonia*) were identified from the root nodules of faba beans grown in two soil types.

This study demonstrates the prospects and importance of faba beans as a key component for sustainable intensification of cereal-based cropping systems in the humid tropical highlands of Ethiopia. However, wider optimal agronomic efficiency of such a legume-cereal system will largely depends on the availability of 1) faba bean germplasm adapted to local biophysical conditions, 2) moderate rates of P fertilizer application and 3) local adaptation of appropriate residue management techniques by local farmers.

# Samenvatting

In de meeste graanteeltsystemen van Afrikaanse tropische hooglanden is de bemesting met fosfor (P) en stikstof (N) beperkt. Het behouden van een voldoende hoge bodembeschikbaarheid van P en N is daarom een belangrijke uitdaging. In deze context zijn biologische stikstoffixatie (BNF), fosfor acquisitie (PAE) en fosfor gebruiksefficiëntie (PUE), kritische factoren voor duurzame landbouwintensivering van tropische hooglanden. Het gebruik van P-efficiënte en $N_2$-fixerende gewassen is een vereiste voor de duurzame intensivering van een op granen gebaseerde kleinschalige landbouw.

Deze studie werd uitgevoerd op landbouwgronden van kleinschalige landbouwers in hoogland teeltsystemen van Zuid-West Ethiopië, die worden gekenmerkt door een combinatie van landbouw en veeteelt en waarin teeltsystemen op basis van granen domineren. Een reeks experimenten werd uitgevoerd om de landbouwkundige prestaties, in termen van graan- en biomasseopbrengst, BNF, PUE en N balans, van *Vicia fab*a te bepalen. Vervolgens werd het effect van gewasresten van geselecteerde *Vicia faba* variëteiten op de opbrengst van tarwe in rotatie onderzocht. Tenslotte werd een follow-up experiment uitgevoerd om te bepalen of klimatologie, bodem of *Vicia faba* variëteit de opbrengst, BNF en PUE van *Vicia faba* bepalen.

Het kwantificeren van BNF van *Vicia faba* door metingen van de natuurlijke aanrijking van $^{15}N$ ($\delta^{15}N$), vereist een juiste bepaling van een zogenaamde B-waarde ($\delta^{15}N$ waarde van *Vicia faba* wanneer volledig afhankelijk van $N_2$ fixatie voor groei). Wij bepaalden experimenteel, variëteit specifieke B-waarden voor zes *Vicia faba* variëteiten en kwantificeerden in hoeverre de variëteit een effect heeft op B-waarden en derhalve op BNF en de N-balans. Het bleek dat B-waarden, gecorrigeerd voor het N-gehalte in de zaden significant verschilden tussen variëteiten

en varieerden tussen 0,5 ± 0,4 en -1,9 ± 1,4 ‰, het geen een variabele isotopenfractionatie tijdens $N_2$ fixatie suggereert. Wanneer de B-waarden, gecorrigeerd voor de N-inhoud van zaden, werden getoetst aan andere benaderingen, viel het op dat BNF-schattingen vertekend konden worden met 34% (15% over- tot 19% onderschatting), wanneer geen rekening werd gehouden met verschillen in variëteit en zaadstikstof. Gegevens over de agronomische prestatie, BNF en PUE van dezelfde zes *Vicia faba* variëteiten werden bepaald. Er was een variëteitverschil ($P < 0,05$) in graan (3,7 ± 0,3 - 5,5 ± 0,6 t ha$^{-1}$) en de totale biomassa opbrengst (7,5 ± 0,8 – 10,8 ± 0,6 t ha$^{-1}$) van de *Vicia faba* variëteiten. Significante verschillen tussen de variëteiten werden ook aangetoond voor BNF (225 - 338 kg N ha$^{-1}$). Variëteiten toonden significante variaties in PUE, maar P applicatie had geen significant effect op de PUE. De variëteit *Moti* toonde de hoogste PUE van 272 kg graan kg$^{-1}$ P; die 1,6-maal hoger was dan de laagste PUE. Dit experiment toonde aan dat variaties in graan- en biomassaopbrengst van *Vicia faba* grotendeels te wijten waren aan verschillen in PUE.

In een volgende stap hebben we onderzocht in hoeverre BNF- en P-efficiënte *Vicia faba* variëteiten leiden tot positieve agronomische effecten op een rotatieteelt met tarwe. Hier werd een eenvoudig input - output model gebruikt voor het berekenen van de N balans en twee mogelijke residubeheer scenario's werden onderzocht.

Scenario I (in de veronderstelling dat alle bovengrondse biomassa wordt geëxporteerd van het veld) gaf een negatieve netto N-balans in het bereik van -78,4 ± 6,1 (variëteit *Degaga*) tot -2,8 ± 8,7 kg N ha$^{-1}$ (variëteit *Moti*) met significante verschillen tussen variëteiten. Scenario II (in de veronderstelling dat alle bovengrondse biomassa, met uitzondering van graankorrels en lege peulen, wordt teruggegeven aan de bodem) toonde aan dat alle balansen sterk werden verbeterd en dat alle *Vicia faba* variëteiten positief N leverden voor de graanrotatie, in de range van 58,7 ± 13,4 (variëteit *Degaga*) tot 174,8 ± 13,7 kg N $ha^{-1}$ (variëteit *Moti*), wat overeenkomt met 126 - 380 kg N $ha^{-1}$ in de vorm van ureum (46% N). De opbrengst van de biomassa en graan van tarwe, geteeld na incorporatie van residuen van *Vicia faba*, verbeterde met 112 en 82%, respectievelijk, in vergelijking met de opbrengst van een tarwe na tarwe teelt. Fosfor toegepast tijdens de groei van *Vicia faba* verbeterde de totale biomassa en graanopbrengst van tarwe, met significant lineair verband tussen de hoeveelheid P toegepast op de *Vicia faba* en tarwe biomassa en graanopbrengst.

Tenslotte werd een meso-cosmosstudie ondernomen met landbouwgronden op twee verschillende hoogtes die onderscheiden zijn in klimaat en bodemkwaliteit, namelijk te *Dimtu* (lagere hoogte, 1790 meter, de gemiddelde jaarlijkse neerslag is 1300 ± 404 mm) en *Dedo* (grotere hoogte, 2160 m, gemiddelde jaarlijkse neerslag is 1874 ± 77 mm), gericht op het beantwoorden van de vraag of het klimaat, de bodem of de *Vicia faba* variëteit de agronomische prestatie, BNF, PUE, rhizosfeer fosfatase-activiteit en de diversiteit van de bacteriën verbonden aan *Vicia faba* wortelknolletjes controleert.

Om het effect op de bodem te kunnen beoordelen, bij elke van de twee locaties, hebben we wederzijds bodem getransloceerd tussen deze twee locaties (*Dimtu* en *Dedo* sites). De opbrengst van graan en biomassa en de nodulatiekenmerken van *Vicia faba*, werden meer bepaald door de plaats (klimaat) dan door de bodem of variëteit. De $N_2$ fixatie was hoger voor *Dedo* ( 1,2 ± 0,1 g N plant $^{-1}$ ) in vergelijking met *Dimtu* ( 0,4 ± 0,0 g N plant $^{-1}$) en de variëteit *Moti* toonde een hogere $N_2$ fixatie ( 0,9 ± 0,2 g N plant $^{-1}$) ten opzichte van *Obse* (1,2 ± 0,1 g N $plant^{-1}$). Verder werd de hoogste PAE door *Vicia faba* bereikt voor *Moti* in *Dedo* bodem op de

*Dedo* locatie ( 99,0 ± 6,0 mg P planten $^{-1}$ ) en de laagste was voor *Obse* in *Dimtu* bodem op *Dimtu* locatie (25,0 ± 3,6 mg P plant $^{-1}$ ), maar de PUE was niet significant verschillend ( $P > 0{,}05$ ). Het bodemtype was belangrijker bij het bepalen van de activiteit van de rhizosfeer fosfatasen dan de locatie of variëteiteffecten. Gebaseerd op 16S rRNA-gen sequencing, werden vijf verschillende geslachten (Rhizobium, Bacillus, Arthrobacter, Staphylococcus en Leifsonia) geïdentificeerd uit de wortelknolletjes van *Vicia faba*, geteeld in twee bodemsoorten.

Deze studie toont de mogelijkheden en het belang van *Vicia faba* aan als een belangrijke component voor duurzame intensivering van teeltsystemen op basis van granen in vochtige tropische hooglanden van Ethiopië. Toch zal een bredere optimale agronomische efficiëntie van dergelijk systeem, grotendeels afhankelijk zijn van de beschikbaarheid van 1) *Vicia faba* germplasma aangepast aan de lokale biofysische omstandigheden, 2) gemiddelde dosis van P bemesting en 3) lokale adaptatie van geschikte residu management technieken door lokale boeren.

# List of symbols and abbreviations

| | |
|---|---|
| %Ndfa | Percentage nitrogen derived from atmosphere |
| °C | Degree Celsius |
| ‰ | Per mil |
| $^{15}N$ | Stable isotope of nitrogen with mass 15 |
| AGRA | Alliance for a Green Revolution in Africa |
| AMF | Arbuscular mycorrhizal fungi |
| ANOVA | Analysis of variance |
| BLAST | Basic Local Alignment Search Tool |
| BNF | Biological nitrogen fixation |
| C | Carbon |
| CEC | Cation exchange capacity |
| CV | coefficient of variation |
| DM | Dry matter |
| EA-IRMS | Elemental Analyzer-Isotope Ratio Mass Spectrometry |
| EMBL | European Molecular Biology Laboratory |
| FNE | Fertilizer nitrogen equivalent |
| GDY | Grain dry yield |
| GLM | General linear model |
| ha | Hectare |
| HI | Harvest index |
| ICARDA | International Center for Agricultural Research in the Dry Areas |
| ISFM | Integrated soil fertility management |
| kg | Kilogram |
| LSD | Least significant difference |
| m.a.s.l | Meters above sea level |

| | |
|---|---|
| MALDI-TOF | Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight |
| Mg | Megagram |
| mg | Milligram |
| NHI | Nitrogen harvest index |
| P | Phosphorus |
| PAE | phosphorus acquisition efficiency |
| PDY | Empty pod dry yield |
| PHI | Phosphorus harvest index |
| $P_i$ | Inorganic phosphorus |
| $P_o$ | Organic phosphorus |
| PRA | Participatory rural appraisal |
| PUE | Phosphorus utilization efficiency |
| R | Correlation coefficient |
| $R^2$ | Coefficient of determination |
| RCBD | Randomized complete block design |
| SE | Standard Error |
| SSA | Sub Sahara Africa |
| T | Ton |
| TC | Total carbon |
| Tg | Teragram |
| TN | Total nitrogen |
| TP | Total phosphorus |
| TSP | Triple super phosphate |
| $\delta^{15}N$ | Natural abundance of $^{15}N$ per mil |

# Chapter 1

# General Introduction

## 1.1. An overview of the Ethiopian setting, soils and cropping systems

Ethiopia is located in east Africa between ca. 3°N and 15°N latitudes and 33°E and 48°E longitudes. It covers an area of ca. 1.1 million km$^2$ and has a considerable variation of climate due to the wide range of altitudes (from 126 meters below sea level to 4620 meters above sea level) (Dejene et al., 2004). The lowlands are arid to semiarid, with annual temperatures of more than 20°C. The highlands, 2500 - 3500m (Moist cold) and 2500 – 3500m (cold), have a temperate climate with annual temperature ranging between 10 and 20°C, while the high mountains (>3500m) have cold, alpine climate with temperature between 10 and 16°C. Annual rainfall increases from 200 mm in the east to over 2000 mm in the southwest (Haileslassie et al., 2005). Though the country is found in the tropics, the highlands encounter both temperate and tropical climates. The wide ranges of topographic, climatic factors, parent material and land use have resulted in extreme variability of soils in Ethiopia. Soils in Ethiopia are largely of volcanic origin. According to the World Reference Base for Soil Resources classification (WRB, 2006) a large proportion of the country's landmass is covered by Lithosols (14.7%), Nitosols (13.5%), Cambisols (11.1%), Regosols (12%) and Vertisols (10.5%). Most of these soils have inherently good physical properties but poor chemical properties and low nutrient stocks due to long-term nutrient mining. In the highlands, the dominating soils are Nitisols, known as the most productive soils of Ethiopia. These soils are acidic, strongly weathered, well drained and have favorable physical properties for agricultural practices. However, Nitisols often have N and P deficiencies, which are the main constraints for cereal and legume production in the Ethiopian highlands (Agegnehu and Tsigie, 2004).

The Ethiopian highlands are one of the hotspots on the African continent with regard to safeguarding food security and in the struggle to protect the natural resource base (Dejene et al., 2004). The highlands cover about 50% of the land area with 95% of the cropping land accounting for over 90% of the country's economy (Gete, 2003). Sustainable food production for the rapidly growing population from a continually reducing farm size (< 1 ha), due to population pressure, is a major developmental challenge in Ethiopia. Population settlement pattern of the highland cropping area is densely populated (ca. 210 people $km^{-2}$) and the lowland cereal based cropping area is sparsely populated (ca. 135 people $km^{-2}$ (CSA, 2010).

The highlands of Ethiopia are characterized by a subsistence crop-livestock mixed farming system where cereal-based cropping systems dominate (Agegnehu et al., 2008). In Ethiopia, crops and cropping systems are diverse due to large agro-ecological and cultural diversity, which in turn has led to variable cropping patterns. Growing crops in mixtures is a common practice in traditional agriculture in various parts of Ethiopia. In mixed farming systems, livestock and crop production are complementary in that the livestock is used for nutrient recycling while crop production provides residues for animal feed (Haileslassie et al., 2005). But at the same time livestock and crop production compete for space.

Cereals (wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; teff, *Eragrostis tef* (Zucc.) Trotter and grain legumes (faba bean, *Vicia faba* L.; field pea, *Pisum sativum* L.;

chickpea, *Cicer arietinum* L. and lentil, *Lens culinaris* Medikus) are major staple food crops cultivated in high altitude areas of Ethiopia (Agegnehu et al., 2006).

Faba bean and wheat are particularly two of the major food crops grown by subsistence farmers under rain-fed conditions in the highlands of Ethiopia. Faba bean is the most important grain legume in Ethiopia in terms of area and production. According to FAOSTAT (2012), the area under faba bean in the main growing season (the rainfed cropping period) was about 0.5 million hectares, making up 35% of the total pulse crops area with a productivity of 1.6 t ha$^{-1}$. It is a major source of protein and a beneficial 'break crop' for improving soil fertility in a cereal-based cropping system. Also, the area under wheat in the main season was about 1.4 million hectares, making up 16% of the total cereal crops area with a productivity of ca. 2.0 t ha$^{-1}$(FAOSTAT, 2012). The introduction of semi-dwarf wheat varieties and increased fertilizer use for the last four decades is a dominant feature of the highland crop agriculture (Agegnehu et al., 2008). However, the increase in input costs, reduction in farm size and soil quality, and increasing problem with buildup of insect pests, diseases and weeds have threatened the ecological and economic sustainability of wheat production in these highlands.

However, single cropping of high-yielding and high fertilizer-input varieties has been promoted for several decades as an approach to enhance food production in the country (Agengnehu et al., 2008). Despite this, a recent resource use study in Northern Ethiopia has shown that farmers have changed their cropping system from growing a pure crop of improved varieties of semi-dwarf wheat to mixed intercropping with a small proportion of faba bean and field pea (Agengnehu et al., 2008). This has attracted attention from research and development stakeholders. According to Agengnehu et al. (2008), the reasons for such a practice was land shortage coupled with the need to produce the cereal as main crop and the legumes as component crops to generate additional benefits, enabling them a spatial crop intensification system under the prevailing arable land scarcity.

Moreover, faba bean-wheat and faba bean-barley rotation is also common feature of cropping systems in most of the Ethiopian highlands, allowing temporal diversification of the agro-ecosystem. In addition to being an important food crop, faba bean also plays an important role in the maintenance of soil fertility in the wheat and barley cropping systems of the Ethiopian highlands because of atmospheric $N_2$ fixation. Research reports indicate that substantial grain yield improvement has been recorded in wheat grown after faba bean (Amanuel and Tanner, 1991; Habtemichial et al., 2007). However in Ethiopia, very little effort has been made to quantify the amount of biologically fixed N (BNF) by faba bean from the atmosphere (e.g., Amanuel et al., 2000) and little is known of the rotational and non-rotational effects (see section 1.5.4) of faba beans in the highland cropping systems of Ethiopia.

## 1.2. Overview and description of the study area

This thesis research was carried out in selected villages of the Gligel-Gibe catchment in south west Ethiopia (Fig 1.1). The catchment has an area of 4225 km$^2$ and lies between 7° 22' 72" - 7° 34' 84" N and 37° 21' 05" - 37° 28' 80" E with an altitude range of 1689-3018 meters above sea level. Geologically, the area is dominated by volcanic rocks of the Eocene and Paleocene, rhyolites, trachytes, rhyolitic and trachytic tuffs, ignimbrites, agglomerates and basalts (Tadesse et al., 2003). Mean annual rainfall in the catchment is ca. 2000 mm, of which around 60% of the rainfall occurs in the rainy season, lasting from April until September while the mean annual air temperature is ca. 19 °C (NMA, 2009). The major soil types in the study area are Nitisols, Acrisols and Vertisols, which are known as the most productive soils of Ethiopia. The Nitisols and Acrisols are strongly weathered, well drained and have favorable physical properties for agricultural practices. However, these soils often have N and P deficiencies, which are the main constraints for cereal and legume production in the Ethiopian highlands (Agegnehu and Tsigie, 2004). In acidic soils, P can be dominantly adsorbed by Al- and Fe oxides/hydroxides. It can be first adsorbed on the surface of clay minerals and Al- and Fe oxides by forming various complexes (Shen et al., 2011). One unique characteristic of P is its low availability due to slow diffusion and high fixation in acid soils (Shen et al., 2011). Based on the classification of LANDSAT satellite image of the year 2006, the land cover classes derived are crop land (42%), grassland/shrub-land (29%), and forest (20%) (data from Kefelegn Getahun, ongoing PhD research at KU Leuven, unpublished).

In order to select the study villages, participatory rural appraisal (PRA) (Bekele et al., 2010) and soil survey (Regassa, 2009) were carried out in the Gilgelgibe catchment (Fig 1.1). Based on the PRA and soil survey results, Dedo (alt. >2000 m) and Dimtu (alt. >1750 m) were selected for the field trials. The description of the sites, land use characteristics and cropping systems are described further.

Continuous cropping is common agricultural practice because many farmers at present cannot expand their land and it is most likely to remain a key feature of the cropping system. The highland mixed farming system is characterized by a very low level of specialization of production. Livestock production is also an integral part of the system. Ownership or access to livestock has a strong influence on farmers' cropping practices. Animal traction (mainly oxen) is used for land preparation to produce cereals and pulses. Livestock possession of households can influence efforts towards land resource management such that those with more livestock possession (≥ 8; at least 2 pairs of oxen, 3 or more cows, a horse and a mule, 4 or more sheep) can manage their farmlands through application of more manure than those with less livestock possession (Bekele et al., 2010). In the highlands, land resource management is also influenced by land tenure and family size. Households with relatively small family size can better manage their lands than those with large family size provided that they have the same size of land. The main reason is that small families have the possibility of fallowing and retaining crop residues on their land to improve soil fertility.

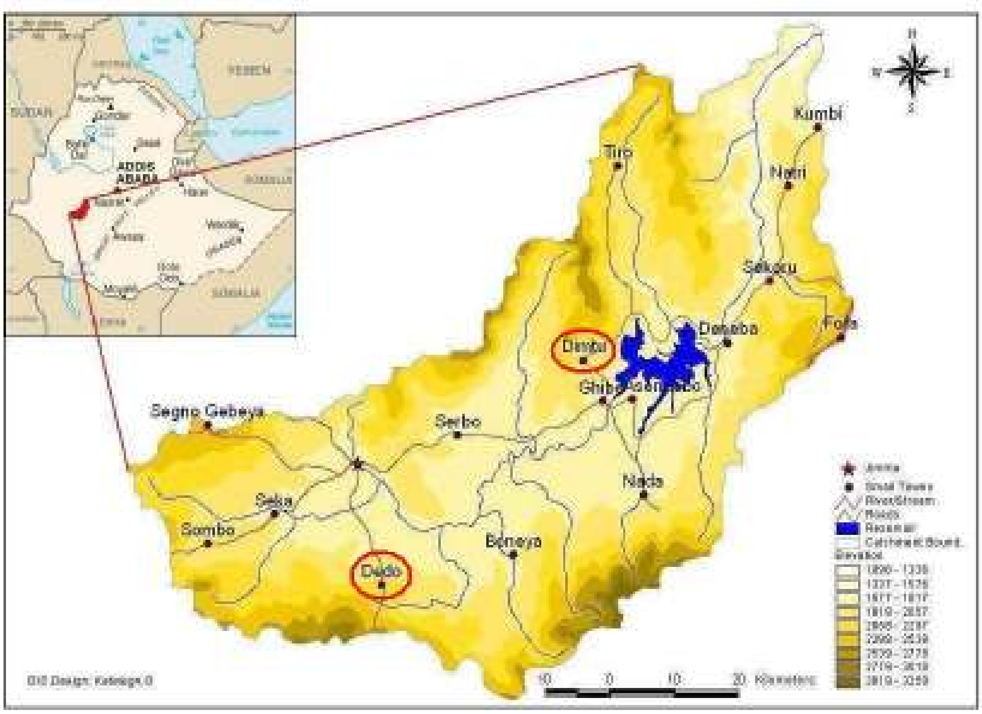

Figure 1. 1. Locations of the study area namely Dedo (higher altitude; 2160 m) and Dimtu (lower altitude; 1790 m) in the Gilgelgibe catchment, southwest Ethiopia.

The main land use in the catchment is arable land. The cropping system at the higher altitude is characterized by cereal (wheat, teff, barley) and legume (faba bean, field peas) production grown on small plots, ranging in size from < 1 ha to over 3 ha in the highland sites. Enset based cereal-legume cropping system can be found on hill slopes and valley bottoms with good vegetation cover (Fig 1.2). Enset (*Ensete ventricosum*) is a banana-like (also called "*false banana*") perennial crop indigenous to Ethiopia. It is usually planted around the homesteads and the plant is used mostly for its starchy pseudostems. In such kind of cropping system of Ethiopia, more nutrient inputs are allocated to enset garden (Haileslassie et al., 2005) because enset crops require more nutrients. Consequently nutrient accumulations are observed in enset garden areas and depletions occur in outfields.

However, most agricultural lands located on the hill slopes are affected by soil erosion and landslides, which has developed into one of the major crop production constraints particularly in the outfields. Low productivity, poor response of crops to chemical fertilizers and the very high cost of chemical fertilizers are key constraints (Bekele et al., 2010). The area at the lower altitudes is also characterized by cereal based cropping system where the major crops are maize, teff and sorghum whereby mono-cropping of these cereals dominates in the area. Topographically, flat lands dominate the lower altitude areas with limited vegetation cover due to removal of natural vegetation for the expansion of cereal cultivation and grazing land. The traditional agriculture of the area is based on a mixed crop-livestock system. Cattle are important for draught power, production of manure to fertilize the soil and as security against emergencies. In the lowlands cattle are grazed on communal range lands and kept in the household pens overnight. In the highland where communal grazing areas have been converted into farm land because of population pressure, feeding on private grass patches, front yards, and road sides is widespread. Crop residues constitute the major source of feed for livestock. In the lowland, cattle graze on crop residues in the field but in the highland residue is collected and brought to cattle housed in pens. With human population growth, agricultural expansion and the widespread conversion of former grazing areas to crop land, crop residues have become increasingly important, both as feed for a farmers own animals and as a commodity to sell to livestock owners. Valley bottoms in particular are subject to increasing competition, with strict rules of grazing management (Elias et al., 1998).

Figure 1. 2. Enset based cropping systems in the highlands of Dedo, Southwest Ethiopia. Enset (*Ensete ventricosum*) is a banana-like (also called “false banana”) perennial crop indigenous to Ethiopia grown around the homesteads as food securty crop.

## 1.3. Soil fertility depletion and nutrient balances

Decline in soil fertility is one of the main constraints of agricultural productivity in Africa (Sanchez and Leakey, 1997; Stoorvogel and Smaling, 1998) because food production in the tropics and subtropics usually depends on available soil nutrient stocks (Sheldrick et al., 2002). Soils with poor nutrient contents, particularly N and P, are widespread in Sub Sahara Africa (SSA), and this has been widely recognized as one of the crucial causes of poor agricultural productivity in SSA. Compared to other parts of the world where the so-called 'agricultural green revolution' has been stimulated by mechanization and high fertilizer use, SSA soil nutrient balances (= input – export via harvest and losses) remain largely negative (Smaling et al., 1997). Despite a generalized trend of decreasing soil fertility in SSA (Stoorvogel et al., 1993), rates of change in soil nutrient stocks differ between farms and fields within farms (Haileslassie et al., 2007; Zingore et al., 2007). Smallholder farmers typically have limited amounts of nutrient resources that are preferentially used on fields closest to homesteads, leading to fertility gradients with increasing distance from homesteads (Zingore et al., 2011).

In Ethiopia soil nutrient depletions and related low agricultural productivity are serious problems. In a study on soil nutrient balances at national and regional level, Haileslassie et al., (2005, 2007) reported large variations in the nutrient balances of different cropping systems, ranging from nutrient accumulating systems (e.g. Enset, *Ensete ventricosum*) to most cereal cropping systems with strongly negative nutrient balances. In most cases, both organic and mineral fertilizer resources are preferentially allocated to the part of the farm used for growing the main food security crop, often close to the homestead, whilst plots further away are neglected. Such management decisions result in creation of gradients of decreasing soil fertility with distance from the homestead (Sanginga and Woomer, 2009). At the national level, Haileslassie et al. (2005) reported nutrient depletion rates of 122 kg N ha$^{-1}$ year$^{-1}$, 13 kg P ha$^{-1}$ year$^{-1}$ and 82 kg K ha$^{-1}$ year$^{-1}$. In southwest Ethiopia, Aticho et al. (2011) studied farm level nutrient depletion rates for highland and lowland cropping systems. Thus, very high nutrient depletion rate (kg ha$^{-1}$ year$^{-1}$) was calculated for N (53 - 58), P (8 - 11) and K (48 - 53) in the highland cropping system, whereas in the lowland cropping system high N (34 - 38) and very high P (7 - 11) and K (23 - 33) depletions were calculated.

Nutrient depletion in Ethiopia has several causes. Application of organic fertilizer like crop residues and manure is limited because of competitive uses (e.g., animal feed and household energy). Also problems in the fertilizer sector have restricted the wider use of inorganic fertilizers. Fertilizer subsidies have been stopped since 1997. Consequently costs of fertilizer escalated. At the same time, low grain prices on the market probably discouraged farmers from using fertilizers. Additionally, inadequate soil conservation practices and reduced fallow periods contributed to the problem (Haileslassie et al., 2005, 2006). Therefore, improved soil fertility management practices need to be embedded within the overall farming practices in order to tackle soil fertility depletions thereby enhances crop productivity. It is less likely that increased crop yields can be achieved without employing improved soil fertility management practices combined with adoption of improved crop varieties (Sanginga and Woomer, 2009). Improved soil fertility management recognizes the application of sufficient amounts of mineral fertilizers, sound agronomic management practices to maintain soil organic matter content, controlling soil erosion and inclusion of legumes in cropping systems among others (Sanginga and Woomer, 2009).

The launching of the Alliance for a Green Revolution in Africa (AGRA), aiming at increasing fertilizer use from the current 8 kg to 50 kg fertilizer ha$^{-1}$ is a recent milestone event (Abuja Fertilizer Summit, 2006) recognizing that sustainable intensification needs to rely on the reasonable use of external nutrient inputs. Since fertilizer is an expensive commodity and because the overuse of fertilizer can lead to adverse environmental side-effects, research and development communities have increasingly adopted integrated soil fertility management (ISFM) as a means for boosting crop productivity through reliance on improved soil fertility management technologies with minimal environmental impacts (Vanlauwe et al., 2011). ISFM is defined as "*the application of a set of soil fertility management practices, that necessarily include the use of fertilizer, organic inputs, and improved germplasm combined with the knowledge on how to adapt these practices to local conditions, aiming at maximizing agronomic use efficiency of the applied nutrients and improving crop productivity*" (Vanlauwe et al., 2010). This definition rather characterizes approaches combining available and locally relevant technologies in a way that increases the agronomic efficiency of individual interventions.

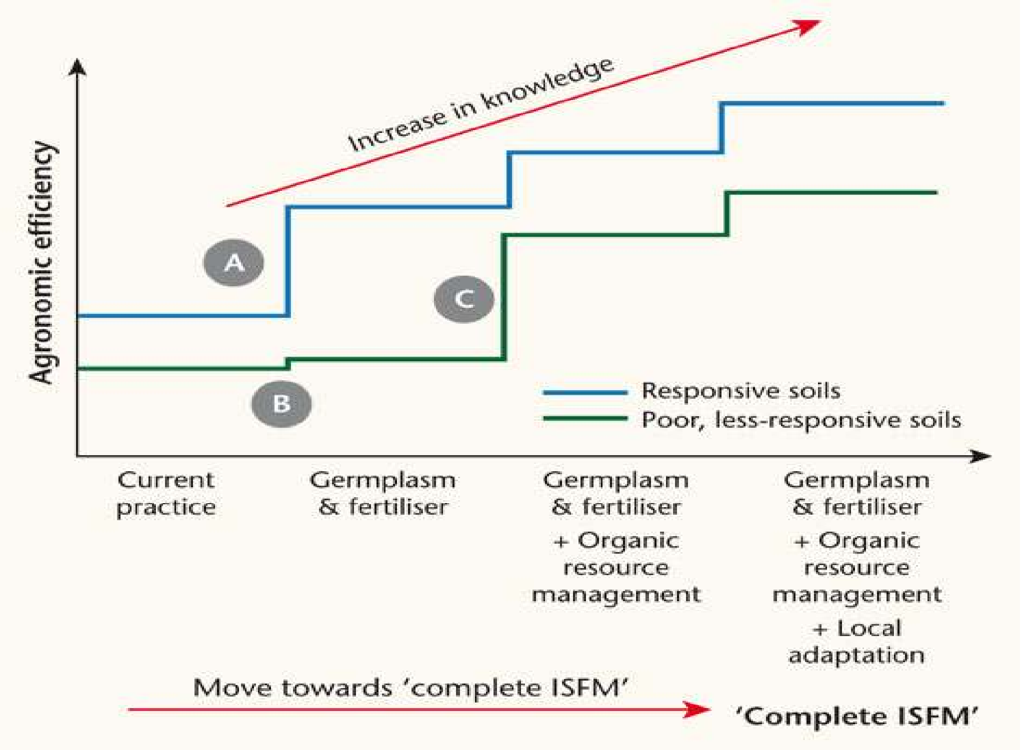

Figure 1.3. The conceptual relationship between the agronomic efficiency of fertilizers and organic resource and the implementation of various components of integrated soil fertility management, culminating in complete ISFM towards the right side of the graph (Adapted from Vanlauwe et al., 2010).

A conceptual framework for ISFM is shown in Fig 1.3 (Vanlauwe et al., 2010) but it should be noted that, by definition, the exact sequencing and combination of interventions will vary by local area; hence this conceptual framework should not be considered as a prescriptive solution. Other aspects of soil fertility management can also be included in ISFM programs, such as soil and water conservation, timing and method of mineral fertilizer application, practices to improve availability, quality, and storage of organic matter, and the maintenance and enhancement of beneficial soil organisms (IFPRI, 2010). There are strong evidences to suggest that ISFM approaches in SSA and Ethiopia have demonstrated yield and economic benefits much greater than the benefits of its single component (e.g., Agegnehu and Chilot, 2009; Oikeh et al., 2010; Sanginga et al., 2003; Vanlauwe et al., 2001).

## 1.4. Sustainable cropping system: concepts and principles

In order to meet the food needs of the ever increasing population, food production worldwide should increase substantially in the coming years and decades (Godfray et al., 2010). However, there are different opinions about how food production should best be achieved. Some propose that agriculture will have to expand into new lands, but this is increasingly unlikely due to the competition for land from other human activities and is also a costly solution. Others propose that food production growth should come through intensified efforts to repeat the approaches of the Green Revolution. What is clear regardless of these differing views is that more will need to be made of existing agricultural land. Traditionally, agricultural intensification has been defined in three different ways: *increasing yields per hectare, increasing cropping intensity (i.e. two or more crops) per unit of land or other inputs (water), and changing land use from low value crops or commodities to those that receive higher market prices* (Pretty et al., 2011). Sustainable agricultural intensification is defined as *producing more output from the same area of land while reducing the negative environmental impacts and at the same time increasing contributions to natural capital and the flow of environmental services* (Pretty et al., 2011).

The major challenge that governments in SSA are facing today is how to achieve food security and reduce poverty, while simultaneously mitigating degradation of important ecosystem services (Teklewold et al., 2013). The low and stagnant returns from African agriculture has been emphasized in the literature (Bluffstone and Köhlin, 2011; Pretty et al., 2011; World Bank, 2007).

Nutrient supply is also a key constraint across African soils and is one of the challenges in sustainable crop production. Nutrient deficiency and low fertilizer use is widespread in many parts of African soils compared with other regions. According to Reij and Smaling (2008), the average use of mineral fertilizers in sub-Saharan Africa is about 8 kg of NPK $ha^{-1}$, compared to a middle and low income country average of about 100 kg $ha^{-1}$. The net removal of nutrients via harvested products increases with an increase in yield unless nutrient cycles are closed (Pretty et al., 2011). Thus, farms in such contexts will need to replenish soil nutrients for sustainable productivity cropping systems. Various approaches have been implemented to sustainably intensify the African agriculture including use of inorganic fertilizers, organics, composts, legumes, and agro-forestry trees systems and combined use of inorganic-organic fertilizer sources (Pretty et al., 2011).

Many ecosystem services such as nutrient recycling, nitrogen fixation, soil fertility restoration, and biological control of pests and diseases are under threat in key African food production systems that are vital for sustainable food security (Teklewold et al., 2013). The causes of environmental degradation in SSA include declining and/or abandonment of fallow periods, inadequate investment in sustainable intensification, lack of diversification and continuous mono-cropping in the traditional cropping systems (Lee, 2005). These trends have contributed to low agricultural productivity and food insecurity in SSA and will continue to do so in the absence of appropriate intervention measures.

The successes of the ‘Green Revolution’ in South Asia and Latin America to boost crop productivity were partially based on the use of improved varieties, water, and fertilizer (Singh, 2000). The Green Revolution in South Asia and Latin America boosted crop productivity through the deployment of improved varieties through breeding, water, and fertilizer. However, efforts to achieve similar results in SSA largely failed (Okigbo, 1987, cited by Vanlauwe et al., 2012) as the input levels required along with the new varieties were too high for poor farmers. On the other hand, there is a call for sustainable intensification and diversification of agriculture in SSA, which has gained support in recent years, especially in densely populated areas where natural fallows are no longer an option. This has been attributed, in part, to the growing recognition that enhanced farm productivity is a major entry point to break the vicious cycle underlying rural poverty (Vanlauwe et al., 2011).

Jensen et al. (2010) have characterized a cropping system in three main factors: (1) the nature of the crops in the system and how they respond to and affect the biological, chemical and physical environment, (2) the succession of crops in the system (from monoculture to species rich dynamic or fixed rotations) and (3) the series of management techniques applied, including crop varieties in the system. To develop successful cropping systems it is therefore necessary to understand how crops such as legumes responds to biological, chemical, physical, and climatic variables, and how this response can be influenced by management. It is also important to determine how legume cultivation affects the productivity of subsequent crops. The challenge is to exploit synergism in time and space through crop sequencing to enhance crop yields with improved resource use efficiency and a reduced risk of negative impacts on the environment via integration of ecological and agricultural sciences (Jensen et al., 2010). The promising research findings on BNF by legumes and their benefit to subsequent crops are also factors, which stimulate interest for the use of faba beans in cropping systems (Jensen et al., 2010).

## 1.5. Role of faba bean in sustainable cropping systems

### 1.5.1. The faba bean (*Vicia faba* L.) crop

Faba bean, also called broad bean or horse bean (Fig 1.4), is native to the Mediterranean basin and has been cultivated for ca. 8-10,000 years (Zohary and Hopf, 2000), and it is an important crop in warm temperate and subtropical areas. It is a significant source of protein rich food in developing countries and is used both as a human food and feed for pigs, horses, poultry and pigeons in industrialized countries (Jensen et al., 2010). As far as animal nutrition is concerned, tannins, vicine, and convicine are faba bean seed constituents, which have been demonstrated in several studies to have an anti-nutritional effect in the diet of monogastric animals (Crépon et al., 2010). Faba bean is most commonly included in the diets of peoples of the Middle East, the Mediterranean region, China and Ethiopia (Jensen et al., 2010). The nutritional value of faba bean is high and in some areas is considered to be superior to peas or other grain legumes (Crépon et al., 2010). However, the presence of chemical compounds like vicine and convicine (C) in the cotyledons may cause health problems in particular situations (Crépon et al., 2010). Faba bean is also grown for green manure and can significantly enhance yields of cereals or other crops.

Faba bean requires a cool season for best development and the growing seasons should have little or no excessive heat, optimum temperatures for production range from 18 to 27°C

(Link et al., 2010). Faba beans grow best on good-structured clay or loam soils with pH 6.5-9, tolerate nearly any soil type (Jensen et al., 2010) and are reasonably tolerant to water-logging. Plants are reasonably tolerant to water-logging, but are more prone to infection from foliar diseases such as Chocolate spot (*Botrytis fabae* Sard.) and rust (*Uromyces fabae* Pers.) (Fig 1.5) under waterlogged conditions. Any factor tending to weaken the crop, such as acid soil, phosphate deficiency and waterlogging of the soil, renders the plant more liable to aggressive infections (Wilson, 2008). Chocolate spot is the most severe disease of faba beans in Ethiopia, with symptoms varying from small brown spots on the leaves to black discoloration of the entire plant. This fungus can cause yield losses up to 60% (Dereje and Yaynu, 2001). The impact of chocolate spot disease can be reduced by proper weeding, early planting, optimal spacing, use of resistant cultivars (e.g., *CS-20DK*) and reduction of waterlogged conditions (Jensen et al., 2010; Sahile et al., 2008).

Figure 1.4. The faba bean plant (a), the pods (b) and grains (c) in the highlands of Dedo, Southwest Ethiopia

Faba bean production is more evenly distributed around the world than most other grain legumes. The cultivated faba bean world area was estimated to be 2.4 million hectares in 2012, with 39% of the total global area of production being located in China followed by Ethiopia, Morocco and Australia with 19, 8 and 7%, respectively (FAOSTAT, 2012). According to FAOSTAT (2012), average grain yield of faba bean in Ethiopia (1.6 t $ha^{-1}$) is far below the Australian (2.6 t $ha^{-1}$), but comparable to China (1.5 t $ha^{-1}$) and greater than that of Morocco (0.8 t $ha^{-1}$), while the world average is 1.7 t $ha^{-1}$. Production has been constrained by several yield limiting factors, which may be attributed to the inherent low yielding potential of local varieties and the inherent low soil pH and associated low N and P availability of soils (Agegnehu and Chilot, 2009; Agegnehu et al., 2006), susceptibility to a range foliar fungal diseases (e.g. Chocolate spot, *Botrytis fabae*; Ascochhyta blight, *Ascochyta fabae*; Cercospora leaf spot, *Cercospora zonata*) that may require fungicide treatment for control, which adds to the production costs (Stoddard et al., 2010).

The production status of faba bean in the above-mentioned countries highlights the role of faba bean as a basic protein food in this part of the world. If major limitations to faba bean yields can be overcome, there is a huge potential worldwide for increasing the occurrence of faba bean in cropping systems.

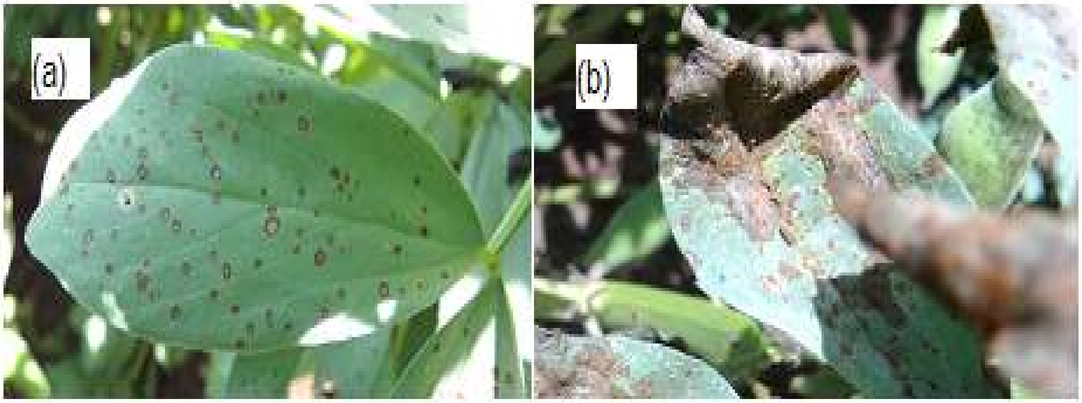

Figure 1.5. Chocolate spot (Botrytis fabae Sard.) (a) and leaf rust (Uromyces fabae Pers.) (b) of faba beans at Dedo, southwest Ethiopia.

### 1.5.2. Biological $N_2$ fixation (BNF) and N balance

Biological $N_2$ fixation (BNF) is a natural process in legume crops, where atmospheric nitrogen ($N_2$) is fixed into ammonia ($NH_3$) in plant root nodules by a symbiotic form of Rhizobia (*Rhizobium leguminosarum* bv. viciae) a gram-negative *Proteobacteria*. The plant assimilates this $NH_3$ into proteins, nucleic acids and other nitrogenous compounds (Strodtman and Emerich, 2009). According to Herridge et al. (2008), BNF from legume crops contributes 2.95 Tg N to agriculture globally. BNF has a high potential for low-input systems, as in large regions of Africa, where more N is removed from the soil than is replenished, which results in depletion of soil nutrients and land degradation (Cocking, 2009).

Any field estimate of $N_2$ fixation input requires an assessment of (a) the percentage of the legume N derived from the atmosphere (%Ndfa) and (b) the total amount of N in legume biomass during the interval of the study (Unkovich et al., 2000). These two components are then multiplied to each other to give the amount of N fixed. By subtraction from total N in the crop, the amount of legume N derived from the soil can be obtained.

As already mentioned, the principal agronomic advantage of faba bean is its ability to fix nitrogen by symbiosis with Rhizobium bacteria, and thereby substantially contribute to the supply of protein for human food and animal feed and greatly reduce dependence on energy consuming mineral N fertilizers (Jensen et al., 2010; Köpke and Nemecek, 2010). In contrast to other legumes, faba bean can maintain high rates of BNF (%Ndfa = ca. 60%) in the presence of high amounts of available N in the soil (ca. 100 kg N $ha^{-1}$) (Hardarson et al., 1991; Schwenke et al., 1998; Turpin et al., 2002), which is attributed to its low rooting density and rooting depth compared with other legumes. Up to 96% of the N taken up by the crop has been measured as nitrogen derived from atmosphere (Ndfa) (López-Bellido et al., 2006). For fertile soils, %Ndfa values between 60 and 80% have been reported several times for temperate regions or with irrigation (Carranca et al., 1999; Peoples et al., 2009b). BNF by faba bean under field conditions range from 15 to 648 kg N $ha^{-1}$ (Schwenke et al., 1998; Köpke and Nemecek, 2010). The high variation may be the result of variation in specific growing conditions, genotypic variations, and variations in the methods used for quantifying BNF.

There is sufficient capacity for BNF to supply the majority of the faba bean N requirements for growth and field data indicate that $N_2$ fixation can support the accumulation of 10-15 t shoot dry matter (DM) $ha^{-1}$ (e.g. Rochester et al., 1998). However, the formation of a functional symbiosis between legume and rhizobia is dependent upon many environmental factors and management practices (see further).

#### *1.5.2.1. Development of the legume-Rhizobia symbiosis*

The faba bean forms nitrogen-fixing root nodules with *Rhizobium leguminosarum* bv. *viciae* and no species other than *Rhizobium leguminosarum* bv. *Viciae* has been reported to nodulate faba bean (Tian et al., 2007; 2008).

In order to form the symbiosis, Rhizobia are dependent on certain compounds (flavonoids, betaines), which are released by the roots of host plants and serve as signaling molecules. These compounds determine the specificity of the plant-host interaction and induce the nod-genes that are required for nodulation (Gage, 2009). The inducing signals from the plant roots are recognized by the bacteria's Nod-protein, a LysR-type transcriptional factor (Lindström et al., 2010). This factor binds inducing or inhibiting compounds, which further inhibits or enhances the transcription of nod-gene promoters in the bacteria (Peck et al., 2006). When the transcription of a nod-gene is enhanced, a protein is encoded, which synthesizes and secretes a Nod factor (lipo-chitooligosaccharide signal) in the bacteria (Dénarie and Cullimore, 1993). This Nod factor triggers the plant responses that are needed for nodulation (e.g., alterations in root hair growth, cell division at infection sites in plant roots and the regulation of plant genes for nodulation) (Gage, 2009). All this results in the development of nodules by the host plant, in which the Rhizobia are present in the symbiosomes of the plant cells. These membrane-bound compartments have a low oxygen concentration, which is required for the activity of nitrogenase, the enzyme responsible for nitrogen fixation (Cocking, 2009). In return for this nitrogen fixation, the nodule receives photosynthesized carbohydrates such as glucose and sucrose. These components are metabolized to organic acids, which are used by the bacteria to derive energy and low potential electrons needed by the nitrogenase enzyme

(Strodtman and Emerich, 2009). Equation 1 shows the reduction of $N_2$ to $NH_3$. This reaction is catalyzed by an enzyme known as nitrogenase.

$$N_2 + 8H_2 + 16ATP \xrightarrow{\hspace{2cm}} 2NH_3 + H_2 + 16ADP + 16P_i \qquad \text{Equation 1}$$

About two weeks after emergence of the faba beans, the first nodules are set. When no other factors are limiting, the faba bean fixes $N_2$ at increasing rate from flowering until pod initiation (Jensen et al., 2010; Vinther and Dahlmann-Hansen, 2005). At maturity, $N_2$ fixation is limited due to carbon competition between nodules and the growing pods of the matured plant (Schulze et al., 2000) hence most $N_2$ fixation measurements are undertaken at late flowering before the sink-source relationship shifts to the development of seed and pods. Nodule formation and functioning requires a lot of energy (ATP), which makes P the main limiting factor for BNF (Leidi and Rodríguez-Navarro, 2000).

Rhizobia are naturally abundant in most agricultural soils, especially in those soils where there has been a history of legume cultivation (Lindström et al., 2010; Zengeni et al., 2006). A soil often contains a wide diversity of Rhizobia populations, of which the presence, density and effectiveness is influenced by the soil pH, soil aggregation, clay content and temperature (Graham, 2009). In soils with pH lower than 5, survival of *Rhizobium leguminosarum* becomes critical (Unkovich et al., 1997). Therefore, Rhizobia may be indicators of soil properties, with high numbers and diversity of Rhizobia indicating soils with consistent soil fertility management and legume cropping (Lindström et al., 2010).

Nodule assessment of *Leguminosae* is often reported in terms of nodule number, nodule position on the host plant's roots and nodule inside color. Schulze et al. (2000) reported that the number of nodules per plant has no effect on $N_2$ fixation per plant. According to Hardarson (1993), the nodules that are positioned on the lower part of the roots can fix more nitrogen during the growing season than nodules that are positioned on the crown part of the roots. The inside color of the nodules can vary from white over pink to dark red. A white inside color indicates a symbiosis where the $N_2$ fixation is not (yet) effective. Dark pink to deep red color, developed by the leghaemoglobin pigment, indicates active $N_2$ fixation (Lindström et al., 2010).

#### *1.5.2.2. Interactions between soil P, fertilizers and BNF*

With low N fertilizer inputs, the availability of soil P is the main factor restricting $N_2$ fixation. This is common in low pH soils and results in a limited growth of Rhizobia, a restricted growth of the nodules (Kihara et al., 2010) and a reduced growth of the plants (Giller, 2001; Kouas et al., 2005; Leidi and Rodríguez-Navarro, 2000). Although nodule growth was diminished at low P availability, Kouas et al. (2005) found that individual nodule biomass and efficiency of $N_2$ fixation was not sensitive to P deficiency. Overall, the effect of P deficiency on plant performance is dependent on the plant’s specific P absorbing capacity and the extent to which nodule growth is hampered. On the other hand, high levels of soil nitrate ($NO_3^-$) induced by the input of N fertilizer and tillage are known to affect biological nitrogen fixation (e.g., Fig 1.6; Jensen et al., 2010). This effect of high N levels cannot be reversed by increasing the P availability (Kihara et al., 2010; Leidi and Rodríguez-Navarro, 2000). Fan et al. (2006) found a decrease in %Ndfa from 64% at 0 kg N $ha^{-1}$ to 40% at 120 kg N fertilizer $ha^{-1}$.

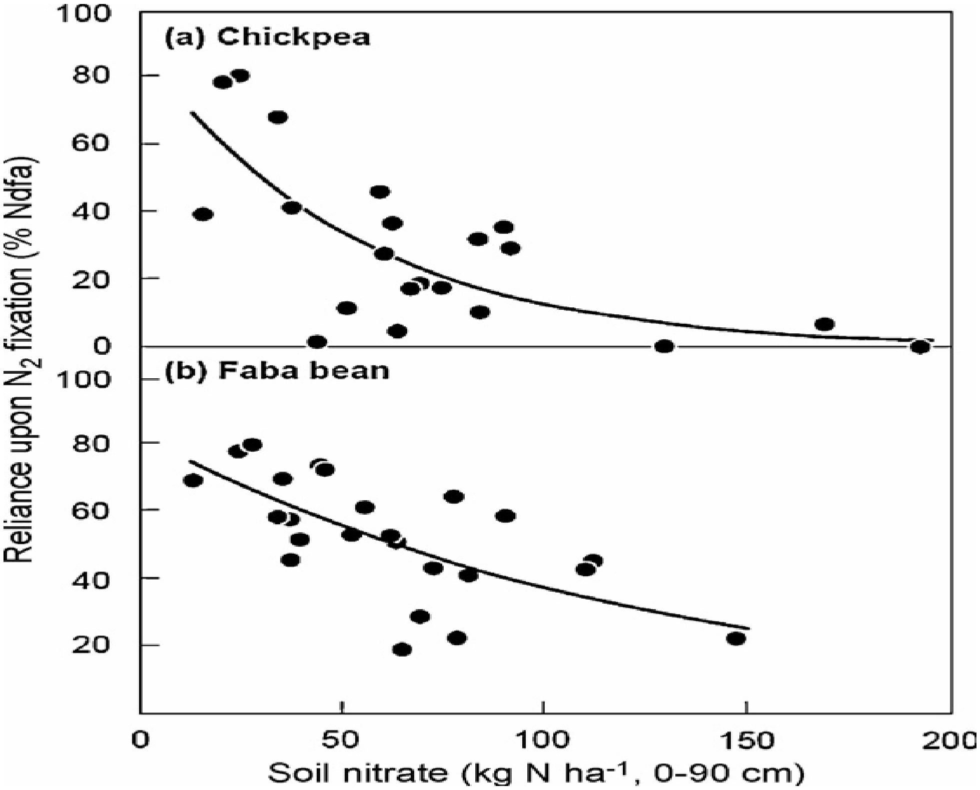

Figure 1.6. The impact of concentrations of soil nitrate at sowing on plant reliance upon $N_2$ fixation (%Ndfa) for growth by farmers' (a) chickpea and (b) faba bean crops in Australia (Adapted from Jensen et al., 2010)

On low available P soils there may be a positive interaction between mycorrhizal activity and $N_2$ fixation by legumes. In soils with low plant-available P, populations of mycorrhizae may play an important role in supporting plant growth by assisting the supply of additional P (Jensen et al., 2010). Cropping systems which include crops of the family *Brassicaceae,* which are not infected by mycorrhizae, may have critically low populations, similar to where a soil is treated with fungicides, or kept bare (George et al., 1994).

### 1.5.3. Methods to quantify BNF

The different approaches that have been used to quantify $N_2$ fixation by legumes have been extensively reviewed (Boddey et al. 2000; Unkovich and Pate 2000; Peoples et al. 2002). This chapter does not intend to provide an exhaustive analysis of all the various applications of those approaches. The principles, assumptions, advantages and disadvantages for each of them are very well discussed in Unkovich et al. (2008) and fall into three broad approaches. The first estimates $N_2$ fixation as the net increase in total N of a plant minus soil N (N balance method). The second aims to separate plant N into the fraction taken up from the soil and the fraction derived from the $N_2$ fixation (N difference, $^{15}N$ natural abundance, $^{15}N$ isotope dilution and ureide methods). The third measures the activity of nitrogenase, the enzyme responsible for $N_2$ fixation (acetylene reduction and hydrogen evolution methods) (Unkovich et al., 2000, 2008). In this thesis, we have adopted the $^{15}N$ natural abundance approach because it depends on the fact that the plant available N in most soils is enriched in $^{15}N$ compared to that in air (Okito et al., 2004). The $^{15}N$ natural abundance method has a number of advantages over other methodologies. It can be applied in greenhouse or field experiments like other techniques, allows $N_2$ fixation to be assessed in almost any situation where both $N_2$ fixing and non-fixing plants are present at the same location and can be easily applied to farmers' fields (Unkovich et al., 2008).

However, one of the potential limitations of the $^{15}N$ natural abundance technique is the need to adjust for isotopic fractionation by the legume during $N_2$ fixation, i.e. the estimation of the so-called '*B-value*' (Doughton et al., 1992; Unkovich and Pate, 2000; Unkovich et al., 2008). Hence the *B-value* is the isotope fractionation corrected atmospheric $N_2$ end member. The *B-*

*value* is defined as the $\delta^{15}N$ value of a legume when completely dependent on $N_2$ fixation for growth (Unkovich and Pate, 2000). The reader is referred to chapter 2 for the description of the technique and chapter 3 for the field application of this method.

### 1.5.4. Effects of faba bean on subsequent crop performance

#### *1.5.4.1 Nitrogen effects*

Growing legumes and incorporating the residues has two main advantages for the smallholder farmers: the legume is produced without fertilizer inputs, and because of mineralization of the legume residues, the subsequent cereal can be grown without or with a reduced amount of phosphorus fertilizers (Evans et al., 1991; Ammanuel et al., 2000; Shah et al., 2003). Faba bean can improve the economic value of a subsequent crop by enhancing the yield and/or increasing the protein concentration of the grain (e.g. Lopez-Bellido et al., 1998, 2006). Increased concentrations of inorganic N in the soil profile after faba bean cropping and increased N uptake by subsequent crops can result from spared N remaining in the soil as a result of a relatively inefficient recovery of soil mineral N compared to other crops and/or the release of N mineralized from above and belowground residues (Rochester et al., 2001; Turpin et al., 2002).

During growth of faba bean, a high amount of $N_2$ is fixed, often resulting in a positive N balance when plant residues are incorporated in the soil after grain harvest. Net N gains due to residue incorporation of about 84 kg N $ha^{-1}$ have been reported (Amanuel et al., 2000, Maidl et al., 1996). As a result of positive pre-crop effects, N fertilization of subsequent crops can be substantially reduced. Pre-crop effects of faba bean can be quantified by yields of subsequent non-legumes compared with those that follow non-leguminous reference crops. Several studies reported savings of up to 100-200 kg N ha$^{-1}$ in the amount of N-fertilizers applied to cereals following faba beans (López-Bellido and López-Bellido, 2001; Jensen et al., 2010). Kirkegaard et al. (2008) found yield increment of up to 20% in wheat following faba bean. Further, fertilizer nitrogen equivalent (FNE) of 108 kg N ha$^{-1}$ for pre-crop faba bean was determined in a temperate climate on a fertile loess soil when compared with unfertilized pre-crops of oats (Köpke and Nemecek, 2010). Wright (1990) reported a similar FNE of about 120 kg N ha$^{-1}$, equivalent to a yield increase of 21% in barley when comparing crop sequences of faba bean-barley-wheat with barley-barley-wheat in Canada. In Ethiopia, Habtemichial et al. (2007) obtained grain yield increases of 36% in faba bean-wheat rotation compared to a barley-wheat rotation. For such a positive effect to occur, the amount of N derived from atmosphere (%Ndfa) by the legumes to the soil should be greater than the amount of N exported in the harvested grain (N harvest index) (Giller and Wilson, 1991).

In low-input systems of less developed countries, crop residues are often removed from the field to be used as animal feed or as a source of energy. This is also the case for the smallholder farmers in the tropical highlands of Ethiopia. Under such conditions, the net N contribution to the soil may be negative. In order to change these practices, farmers need to be convinced that plant residues have more benefits when used as source of nutrients (Bakht et al., 2009; Giller, 2001).

#### *1.5.4.2 Phosphorus effects*

Providing farmers with genotypes adapted to specific conditions of phosphorus availability in the soil and having good phosphorus use efficiency can improve yields, without excessively increasing production cost or damaging the environment. Large genotypic differences in respect of P acquisition and utilization efficiency were reported for faba bean (Stelling et al., 1996; Daoui et al., 2012). The variation for P acquisition and utilization efficiency may arise from differences in root architecture and physiology and/or on biomass translocation and phosphorus partitioning. Varietal differences in P utilization may be attributed to variations in efficient re-translocation and re-use of the stored P in plants (Wang et al., 2010). Therefore, selection of efficient P utilizing crops that yield well in soils with low available P is a cost-effective way of improving crop yields in low-input farming systems (Rose et al., 2011; Rose and Wissuwa, 2012).

Certain legume crops, including faba beans, exude particularly large amounts of organic acids into the rhizosphere, especially as a response to P deficiency (Neumann and Römheld, 1999; Veneklaas et al., 2003; Nuruzzaman et al., 2006). Under such conditions, these exudates can help mobilize P from soil-P pools, which are unavailable to plants that do not possess this adaptation (Nuruzzaman et al., 2006). Phosphorus in the soil is present in different inorganic and organic forms, but plants absorb it as inorganic P (Yang and Jac*obse*n, 1990). Organic phosphorus compounds are important sources for plant P, but they must be mineralized into inorganic form before P can be taken up by plants (Vance et al., 2003). Organic P comprises 30–80% of total P in most agricultural soils. Legumes can use organic P fractions in addition to inorganic P which is due to the release of phosphatases (Adams and Pate, 1992).

There is also evidence that enhanced P availability is partly responsible for the positive effect of legumes on subsequent crops. Kamh et al. (1999) demonstrated that growth and P uptake of a subsequent maize crop were enhanced due to mobilization of soil P by P-efficient legume crops. Hocking and Randall (2001), in a pot experiment, demonstrated a carryover P benefit to sorghum and wheat when grown after white lupin, which exuded large amounts of organic acids. Horst et al. (2001), in a field trial, observed a positive rotational effect of P-efficient leguminous crops on the less P-efficient cereal crops. However, the mechanisms determining P-efficiency in grain legumes, and the extent to which these influence P uptake of subsequent crops was not clearly understood (Nuruzzaman et al., 2005).

Faba bean residues may cause a long-term and significantly higher pH increase compared with wheat residues. Nuruzzaman et al. (2005) have shown that faba bean improved P availability to the subsequent wheat, which has led to better growth in soils where P availability was the main limiting factor. According to the same authors, wheat following faba bean produced more biomass than wheat after wheat and also had higher plant P concentrations, particularly when the faba bean had received P fertilizer. The simultaneous increase in growth and P concentrations indicates that the faba bean has enhanced the bioavailability of P for uptake by wheat. This rotational benefit is presumed to be closely related to the mineralization of P-rich plant residues (Horst et al., 2001). Further, Rose et al. (2010) concluded that growth of wheat following P-efficient faba bean genotypes on acid and alkaline soils was greater than wheat growth after P-inefficient faba bean genotypes, which suggested that utilizing variations in P efficiency of faba beans may further enhance the benefit of faba beans in increasing the P efficiency of cropping systems.

P requirement of faba bean is high because of the strong ATP requirements for nodule formation and functioning (Ribet and Drevon, 1996). Like other legumes, faba bean is well adapted to acquire P from low P soils, especially when compared to cereals (Bolland et al., 1999). $N_2$ fixing faba bean absorbs more cations than anions. Protons are released to balance the plant's internal charge balance, thereby leading to soil acidification in the rhizosphere and enhanced mobilization of soil phosphorus (Köpke and Nemecek, 2010). Also, by exudation of carboxylates, predominantly malate, faba bean can make residual phosphorus available that otherwise would remain fixed (Nuruzzaman et al., 2005) and may indirectly make more phosphorus and potassium available for subsequent crops.

The availability of P in the rhizosphere is influenced by changes in pH and root exudates, which can directly or indirectly affect nutrient availability and/or microbial activity (Richardson et al., 2009; Zhang et al., 2010; Shen et al., 2011). Acidification of the rhizosphere in response to P deficiency has been demonstrated for a number of species (e.g., Hinsinger et al., 2003) and can modify the solubility of sparingly soluble inorganic P compounds and the subsequent availability of phosphate and various micronutrients in soil solution (Richardson et al., 2009). Secretion of phosphatase enzymes into the rhizosphere is also a universal response by plants to P deficiency (Vance et al., 2003) and the activity is significantly greater in the rhizosphere and is considered to be a general response of plants to mobilize P from organic forms.

#### *1.5.4.3 Non-nitrogen pre-crop effects*

When the bulk of the above ground biomass of the legume component is removed from the system, net N contribution to the soil may be negative and so yield increase in the subsequent cereal crop, if any, must be caused by other rotational effects. Such rotational effects have been observed for several legumes. Sanginga et al. (2002) recorded yield increase in maize following soybean and Habtegebrial and Singh (2006) in wheat following field pea where the N balances in both cases were -8 kg $ha^{-1}$ and -9 kg $ha^{-1}$, respectively, suggesting that rotational effects other than N are operating. Faba bean can also provide a range of other potential rotational benefits that are not directly related to N such as reductions in the incidence of grassy weeds, reductions in diseases or pests, improvements in soil structure (Rochester et al., 2001; Kirkegaard et al., 2008; Jensen et al. 2010), favorable microbial community in the rhizosphere (Köpke and Nemecek, 2010). Faba bean is known to be able to suppress inoculum of soil-borne disease cycles within cereals such as take-all (*Gaeumannomyces graminis*), and the effect appears to be similar to the effect of other legume and non-legume break-crops (McEwen et al., 1989).

## 1.6. Aims and outlines of the thesis

This thesis aims at a better understanding the role of faba beans in highland cropping systems of southwest Ethiopia where resources are limited and which are characterized by a subsistence-oriented mixed crop-livestock farming system wherein cereal-based cropping systems dominate. In particular, it aims at achieving better insight in the importance of BNF, N-balance, P utilization efficiency and rotational effects of faba beans.

In Chapter 2, we experimentally determined specific parameters of six faba bean varieties that could potentially affect a correct quantification of BNF, N balances and showed to what extent a variety has an effect on BNF and N balances. The six varieties were selected because they showed good adaptation potential to the prevailing climate and soil conditions, effective nodulation with local rhizobia and good agronomic performance among 15 accessions screened in a previous screening trial at two altitudes in the study area. Further, Chapter 3 presents data on agronomic performance, BNF and also assesses the P acquisition and utilization efficiency of the same six faba bean varieties. We assume that identification and use of such varieties in the current cropping systems could improve N and P nutrition of the legume and possibly also the non-legume crop in a rotation. The variety specific characteristics determined in chapter 2 were practically applied in chapter 3 to quantify BNF via the $^{15}$N natural abundance method. Chapter 4 examines to which extent high BNF and P efficient faba bean varieties identified in Chapter 3 lead to positive agronomic effects on wheat grown in rotation. Here, a simple input-output model was used to calculate the N balance and two possible residue management scenarios are examined. In Chapter 5, a mesocosm study was undertaken on farmers' fields at two different altitudes (Fig 1.1) which are distinct in climate and soil type, namely at Dimtu (lower altitude; 1790 m) and Dedo (higher altitude; 2160 m) aiming at assessing whether climate, soil, or variety control BNF, P acquisition and use efficiency. Finally, the results of chapters 2 to 5 are summarized and more general conclusions and future lines of work are highlighted in chapter 6.

# Chapter 2

# Importance of variety specific *B-value* to quantify biological $N_2$ fixation of faba beans via $^{15}N$ natural abundance

After: Amsalu Nebiyu, Dries Huygens, Hari Ram Upadhayay, Jan Diels and Pascal Boeckx. Importance of correct *B-value* determination to quantify biological $N_2$ fixation of faba beans (*Vicia faba* L.) via $^{15}N$ natural abundance. Biol Fertil Soils, *in press* DOI 10.1007/s00374-013-0874-7

## Abstract

Quantifying biologically fixed nitrogen (BNF) by legumes through the $^{15}N$ natural abundance techniques requires correct determination of a so-called *B-value*. We hypothesized that significant variations in *B-values* exist between faba bean (*Vicia faba* L.) varieties having consequences for BNF and N balance calculations. We experimentally determined *B-values* for a range of faba bean varieties and quantified to what extent variety has an effect on *B-values* and hence BNF quantification. Seeds of six faba bean varieties released in Ethiopia were inoculated with *Rhizobium fabae* strain LMG 23997-19 and grown in vermiculite with an N-free nutrient solution in a growth room until full flowering. Total N and $^{15}N$ content of nodules, roots and shoot components was analyzed separately to determine the weighted whole plant $^{15}N$ fractionation during $N_2$ fixation, i.e. the *B-value*. Owing to its large seed size and high N content, a correction for seed N was carried out. We then calculated the percentage of N derived from air (%Ndfa), BNF and N-balance for faba beans grown in the field using three *B-value* scenarios (variety specific *B-value* corrected for seed N, variety specific *B-value* without seed N correction and a literature derived *B-value*). Whole plant seed N corrected *B-values* were significantly different (P <0.05) between varieties and varied between +0.5 ± 0.4 to -1.9 ± 1.4‰ suggesting a variable isotope fractionation during $N_2$ fixation. The %Ndfa was significantly (P < 0.05) different between varieties (59 ± 4.2 – 84 ± 4.5%) using seed N corrected *B-values*. BNF (218 ± 26.2 - 362 ± 34.7 kg N $ha^{-1}$) was significantly (P < 0.05) different between varieties for corrected and uncorrected *B-values*. Soil N balance did not result in statistically significant (P > 0.05) difference between varieties for all three *B-value* scenarios. Use of inappropriate *B-values* masked the difference between varieties and affected their ranking in terms of BNF, resulting from an over- to underestimation of 15 and 19%, respectively. When applying the $^{15}N$ natural abundance technique to compare BNF of legume accessions, we recommend determining a *B-value* for each accession. For legumes with large seeds such as faba beans it is moreover essential to account for seed N when determining the *B-value*.

Key words: faba bean, *B-value*, $^{15}N$ natural abundance

## 2.1. Introduction

Atmospheric nitrogen (N) fixation in crop and pasture legumes plays a key role in providing human and livestock protein and for maintaining soil fertility in agro-ecosystems (Unkovich et al., 2010). Improving N fertilizer efficiency and exploitation of biologically fixed N (BNF) are thus of great importance for long-term sustainability of crop production in agro-ecosystems (Unkovich et al., 2008). The global high price for N fertilizer and the overall environmental impact of excessive fertilizer use (Chianu et al., 2011; Fan et al., 2006) warrant a growing interest in legume BNF, especially for smallholder farmers in the tropics. Many literature reviews document on the magnitude and potential benefits of BNF by legumes within different agro-ecosystems (Herridge et al., 2008; Peoples et al., 2009a; Salvagiotti et al., 2008; Unkovich and Pate, 2000). The use of legume-cereal crop rotation systems, particularly with faba beans, has proven to be an efficient cultivation method to reduce N fertilizer use in tropical highlands (Amanuel et al., 2000; Maidl et al., 1996), and is thus a sustainable option for agricultural intensification. Faba bean is one of the best crop species for atmospheric $N_2$ fixation, with global annual BNF inputs estimate at around 0.3 Tg year$^{-1}$ (Herridge et al., 2008). Research reports also indicated a substantial grain yield improvement for wheat cultivated in rotation with faba beans (Amanuel et al., 1991; Habtemichial et al., 2007; Lopez-Bellido et al., 2006). Nevertheless, in order to improve the N use efficiency of faba bean based cropping systems, an accurate quantification of the proportion of N derived from the air (%Ndfa) is required for the range of faba bean varieties.

The correct estimation and quantification of BNF by legumes depends on the applied methodology (Hardarson et al., 1993). Stable isotope methods have emerged as one of the more powerful tools to advance the understanding of relationships between plants and their environment (Dawson et al., 2002). The natural abundance of $^{15}$N ($\delta^{15}$N) can be used to assess BNF in field conditions without the additional cost and effort of applying $^{15}$N-enriched fertilizer (e.g. Houngnandan et al., 2008; Pate et al., 1994; Okito et al., 2004). This technique depends on the fact that the plant available N in most soils is enriched in $^{15}$N compared to that in air (Okito et al., 2004); hence a simple two end member isotopic mixing model can be used to quantify the contribution of atmospheric N and soil N to a legume crop. The $^{15}$N natural abundance method has a number of advantages over other methodologies. It can be applied in greenhouse or field experiments like other techniques, allows $N_2$ fixation to be assessed in almost any situation where both $N_2$ fixing and non-fixing plants are present at the same location and can be easily applied to farmers' fields (Unkovich et al., 2008).

Since the legume growing in the field acquires N from two end members (soil N and air N), it is required to determine the $^{15}N$ abundance of both the N derived from the soil and that derived from air through fixation. The most reasonable measure of the $\delta^{15}N$ value of soil derived N within the legume plant is to rely on the $\delta^{15}N$ value of a non-$N_2$ fixing reference plant grown on the same soil (Shearer and Kohl, 1988). However, this measure may be subject to errors and the problems associated to reference plant selection have been reviewed earlier in detail (Boddey et al., 2000; Handley and Scrimgeour, 1997; Hogberg, 1997) and is therefore not the focus of this paper. The other potential limitation of the $^{15}N$ natural abundance technique is the need to adjust for isotopic fractionation by the legume during $N_2$ fixation, i.e. the estimation of the so-called '*B-value*' (Doughton et al., 1992; Unkovich and Pate, 2000; Unkovich et al., 2008). Hence the *B-value* is the isotope fractionation corrected atmospheric $N_2$ end member. The *B-value* is defined as the $\delta^{15}N$ value of a legume when completely dependent on $N_2$ fixation for growth (Unkovich and Pate, 2000). Under these conditions legume shoot N is normally depleted in $^{15}N$ relative to atmospheric $N_2$ due to isotope discrimination within plant tissues. Since the *B-value* varies with species, plant age at harvest, growing conditions (Unkovich and Pate, 2000) and seed size (Lopez-Belido et al., 2010) a single *B-value* could not be satisfactory for all legumes and environments. Reported estimates may thus vary considerably, even for the same plant species (Boddey et al., 2000). The *B-values* cited in the literature show a wide range for different legumes (Boddey et al., 2000; Peoples et al., 2009b), and for faba beans specifically

(Fan et al., 2006; Lopez-Bellido et al., 2010; Unkovich et al., 2008). The majority of the *B-values* for legumes usually lie in between 0 and -2.0‰, with one or two exceptions (Okito et al., 2004).

Large errors in $N_2$ fixation calculation can be generated by using incorrect *B-values*, especially when %Ndfa is higher than 85% (Unkovich and Pate, 2000). Doughton et al. (1992) designed a method to estimate *B-values* with plants grown in the field by the combined use of $^{15}N$ enrichment techniques and natural abundance methodologies on the same crop at the same site. Their method was basically a process of adjusting *B-values* until %Ndfa measured by natural abundance best matches %Ndfa as derived from a $^{15}N$ tracer experiments. However, this type of *B-value* estimation technique may not be suitable for BNF measurements when dealing with large number of varieties of a single legume species. An alternative methodology to determine *B-values* is based on the cultivation of N-fixing plants that are grown in N-free growth medium (Vincent, 1970).

Current estimates of *B-values* (e.g. Boddey et al., 2000; Doughton et al., 1992; Houngnandan et al., 2008; Key-Boahen et al., 2002; Lopez-Bellido, 2010; Nguluu et al., 2001; Okito et al., 2004) are often biased due to two reasons. First, *B-values* are typically calculated based on aerial tissues because of ease of sampling. However, such sampling protocols do not take into account the non-uniform distribution of $^{15}N$ between roots, nodules and shoot tissue (Boddey et al., 2000). Second, in order to obtain the true *B-value* an additional adjustment for seed N at sowing should be considered. The significant impact of seed size has often been overlooked in studies of *B-value* and BNF determination. Seed N can constitute a significant proportion of total N accumulated by the crop, especially for large seeded legumes with high seed N contents like faba beans (Lopez-Belido et al., 2010; Okito et al., 2004). In this study, we test the importance of considering the non-uniform distribution of $^{15}N$ in legumes, the effect of variety and the seed N contribution for calculating *B-values* for a range of faba bean varieties. It is hypothesized that significant variations in *B-values* exist between faba bean varieties and that %Ndfa calculations show large variations dependent on the methods applied to calculate *B-values*. Most *B-values* for faba beans reported in the literature are determined using above ground biomass only and did not take into account the seed N and seed $^{15}N$ contribution. We experimentally determined *B-values* for a range of faba bean varieties taking into account (1) the non uniform distribution of $^{15}N$ both in the above and below ground biomass (2) the need for an additional adjustment for seed N and seed $^{15}N$ at sowing; and quantified the effect of variety on *B-values* and hence BNF estimates. We also examined the need for seed N correction for correct *B-value*, hence BNF estimation.

## 2.2. Materials and methods

### 2.2.1 Experimental setup

In order to determine *B-values* of faba bean varieties a hydroponic culture without N sources was setup using a modified Leonard jar system (Vincent, 1970), consisting of a bottle (330 ml) with the bottom portion cutoff and inverted into a 1L Mason jar. A cotton lamp wick was inserted through the neck of the inverted bottle, which was placed on the bottom of the Mason jar. A foam plug in the neck of the inverted bottle held the wick in place. The assembled system was autoclaved (120°C) during one hour and allowed to cool for 24 hours before seed sowing. The inverted bottle was filled with washed and autoclaved (60 minutes) N-free vermiculite.

Six faba bean varieties (*CS-20DK*, *Degaga*, *Gebelcho*, *Moti*, *Obse* and *Walki*) (Table 2.1) were used. These varieties were selected because they had good adaptation potential and agronomic performance for the humid tropical highlands of Ethiopia (Nebiyu et al., 2010). The seeds of each variety were surface-sterilized with 10% $H_2O_2$ for 30 minutes and washed and rinsed 5 times with distilled water. The seeds were then germinated for 3 days on a petri dish with deionized water in the dark at 28 °C and sterile conditions (Rodriguez-Navarro et al., 2000). The germinated seeds were inoculated with *Rhizobium fabae* LMG 23997 ($3.5 \times 10^9$ CFU $mL^{-1}$) by dipping them in viscous *Rhizobium* containing specific growth medium (Vincent, 1970) for 30 minutes. Two inoculated seeds were sown in each jar by carefully punching a hole through the rooting medium (vermiculite) and placing the seed into the vermiculite with sterilized forceps. The experimental design used was completely randomized design (CRD) in six replicates, each jar being a replicate. The seedlings were thinned to one after four days of seedling emergence. Each Mason jar was watered with Norris modified N free nutrient solution (Norris and Date, 1976) containing (per liter of de-ionized water) $KH_2PO_4$ (0.27 g), $K_2SO4$ (0.35 g), $CaSO_4.2H_2O$ (1.0), $MgSO_4.7H_2O$ (0.25 g), $H_3BO_3$ (4.0 mg), $MnCl_2.4H_2O$ (0.99 mg), $ZnSO_4.7H_2O$ (0.58 mg), $CuSO_4.5H_2O$ (0.125 mg), $FeCl_3.6H_2O$ (5.4 mg) and $Na_2MoO_4.2H_2O$ (0.1 mg).

### 2.2.2 Plant growth, harvest and analyses

The plants were grown in a growth room with a 13-hours day length and mean day and night temperatures of about 24 °C and 16 °C, respectively (Fig 2.1). The nutrient solution in the Mason jar was replaced every six days by 250 to 300 mL of the Norris modified N free nutrient solution. The plants were harvested at flowering (47 days after sowing) all at the same time. All of the senescent leaves lying on the media of each jar were periodically collected and kept until harvest, and added to the shoot fraction (aerial portion). The aerial portion, root and nodules were separated during the plant harvest and dried at 65 °C for 72 hours to determine the dry matter. The plant parts were ground using a centrifugal mill (Retsch ZM 200, Germany), using a 0.5 mm sieve. The N concentration, total N and $^{15}N$ of the seed at sowing, and of the shoot, root and nodules were determined via Elemental Analyzer Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). The $^{15}N$ natural abundance ($\delta^{15}N$) was calculated according to the following formula (Högberg 1997):

$\delta^{15}N$ (‰) = $[(^{15}N/^{14}N_{sample} - ^{15}N/^{14}N_{standard})/ (^{15}N/^{14}N_{standard})] \times 1000$; where, the standard is air (Eq. 1).

Figure 2.1. The faba bean plants grown in a growth room in an N free nutrient solution using vermiculite as rooting medium.

### 2.2.3 Correction for seed N

Owing to its large seed size and high N content (Table 2.1), faba bean seed N can constitute a significant proportion of total N accumulated in the plant (Lopez-Belido et al., 2010). Therefore, it was necessary to determine the $\delta^{15}$N of the seed N, discounting its excess $^{15}$N content (using a mass balance) to estimate the *B-value*. This correction was made using the following formula given by Högberg et al. (1994):

$\delta^{15}$N corrected = [(whole plant N × $\delta^{15}$N whole plant) – (seed N × Ps × $\delta^{15}$N seed)]/(whole plant N - seed N) (Eq. 2),

where, $\delta^{15}$N corrected indicates the correction for seed N and Ps is the proportion of the seed N that was incorporated in the plant tissue. According to Okito et al. (2004), Ps is assumed to be 0.5 when correcting shoot tissue only (i.e. 50% of the seed N was incorporated into the aerial tissue) and when correcting for the whole plant Ps is assumed to be 1.

The corrected *B-values* of each variety were then used to calculate %Ndfa, BNF, and soil N balance using data from unpublished $^{15}$N measurements collected from a faba bean experiment with the same varieties carried out on a farmer's field at Dedo (7°28'48" N and 36°52'19"E and at an elevation of 2,160 m above sea level), Southwest Ethiopia (see chapter 3 for site description). The %Ndfa was calculated as follows (Peoples et al., 2009b) with wheat as the reference plant. Wheat and barley have similar duration of growth and nutrient uptake pattern as faba beans and hence they are frequently used as reference crops for faba beans (e.g., López-Bellido et al., 2006; Peoples et al. 2009b).

% Ndfa = 100 x ($\delta^{15}N_{\text{reference plant}}$ - $\delta^{15}N_{\text{legume}}$) / ($\delta^{15}N_{\text{reference plant}}$ – *B-value*) (Eq. 3).

Where, $\delta^{15}N_{\text{reference plant}}$ and $\delta^{15}N_{\text{legume}}$ are the $\delta^{15}N$ values of whole plant wheat and faba bean, respectively.

Table 2.1. Main varietal and seed characteristics of six selected faba bean varieties used in the present study

| | ------Variety characteristics*------ | | | | | | ------Seed characteristics**------ | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variety | Pedigree | Source | Days to flowering | Days to maturity | Grain yield potential (Mg ha$^{-1}$) | Adaptation zone ($^{b}$m a.s.l.) | Seed weight (mg) | N-concentration (mg N kg$^{-1}$ DM) | Total N (mg seed$^{-1}$) | $\delta^{15}$N (‰) |
| *CS-20DK* | CS20DK | Ethiopia | 57-67 | 145-160 | 1.5-3.0 | 2300-3000 | 537 | 4.2±0.5 | 22.7 | -0.5±0.2 |
| *Degaga* | R878-3 | ICARDA[a] | 45-62 | 116-135 | 2.0-4.5 | 1800-3000 | 662 | 4.0±0.6 | 26.6 | -0.9±0.1 |
| *Moti* | ILB4432 x Kuse 2-27-33 | ICARDA | 43-65 | 108-165 | 2.3-3.5 | 1800-3000 | 587 | 3.4±1.1 | 20.3 | -0.6±0.1 |
| *Gebelcho* | Tesfa x ILB4726 | ICARDA | 51-69 | 103-167 | 2.0-3.0 | 1800-3000 | 757 | 3.5±0.5 | 26.7 | -0.6±0.1 |
| *Obse* | CS20DK x ILB4427 | ICARDA | 43-65 | 87-166 | 2.1-3.5 | 1800-3000 | 897 | 4.3±0.9 | 39.2 | -0.3±0.1 |
| *Walki* | Bulga-70 x ILB4615 | Ethiopia/ICARDA | 49-61 | 133-146 | 2.0-4.2 | 1800-2800 | 755 | 3.9±0.2 | 29.7 | -1.1±0.2 |

* Data from MoARD (2009); ** own data

[a] = *International Center for Agricultural Research in the Dry Areas, Aleppo, Syria*

[b] = meters above sea level,

The amount of $N_2$ fixed by the legume (BNF, kg N $ha^{-1}$) = total N yield × (%Ndfa)/100. The N balance (kg N $ha^{-1}$) was calculated as whole plant BNF + seed N at sowing minus N exported via grain N. We compared Ndfa, BNF and N-balance obtained via variety specific seed N corrected *B-values*, variety specific *B-values* without seed N correction and a mean of previously published shoot derived B values (-0.85‰) from literature (Lopez-Bellido et al. 2010; Fan et al. 2006; Unkovich et al. 2008)

### 2.2.4 Statistical analysis

All data were subjected to analysis of variance (one-way ANOVA) using the general linear model (GLM) procedure of Statistical Analysis System (SAS) software version 9.2 (SAS, 2008). All the data were first checked for normality using the Proc univariate, option Normal procedure of SAS. Quantile-Quantile plot (Q-Q plot) on the residuals from ANOVA was taken into consideration for assessing the normality of the residuals. When the F-test denotes significant effect, the least significant difference (LSD) was used to compare treatment means at $P < 0.05$. In a balanced design like CRD in which the same number of observations contributed to each mean, the LSD is more appropriate for mean comparison (Webster, 2007).

## 2.3. Results

### 2.3.1 Dry matter yield

The nodule, root and shoot dry matter yield was significantly different between the faba bean varieties (Table 2.2). The average whole plant dry matter yield of faba bean varieties was 2.4 g $plant^{-1}$ with significant difference between varieties varying from 1.6 ± 0.1 g $plant^{-1}$ (*Gebelcho*) to 2.9 ± 0.2 g plant$^{-1}$ (*Obse*). Varieties *CS-20DK*, *Moti* and *Walki* produced similar ($P > 0.05$) whole plant total dry matter yield which was comparable with *Obse* (Table 2.2).

Table 2.2. Dry matter yield (mean ± SE) of faba bean plant parts (shoot, root, nodule and total plant) of six varieties harvested at flowering (47 days after sowing) grown in N free nutrient medium. Means followed by different letters in a column are significantly different at $P \leq 0.05$

| | Dry matter (g plant$^{-1}$) | | | |
|---|---|---|---|---|
| Variety | Shoot | Root | Nodules | Whole plant |
| *CS-20DK* | 1.9±0.1ab | 0.7±0.1ab | 0.06±0.01ab | 2.6±0.1ab |
| *Degaga* | 1.5±0.2b | 0.5±0.04bc | 0.08±0.01a | 2.1±0.3b |
| *Gebelcho* | 1.1±0.2c | 0.5±0.07bc | 0.02±0.01b | 1.6±0.1c |
| *Moti* | 2.0±0.1a | 0.8±0.05a | 0.05±0.02ab | 2.9±0.2a |
| *Obse* | 1.9±0.1ab | 0.7±0.06a | 0.09±0.02a | 2.7±0.1a |
| *Walki* | 1.8±0.1ab | 0.7±0.05ab | 0.09±0.02a | 2.6±0.2ab |
| Average | 1.7 | 0.6 | 0.07 | 2.4 |
| $LSD_{0.05}$ | 0.4 | 0.2 | 0.04 | 0.5 |

### 2.3.2 N concentration

The average total N concentration (mg kg$^{-1}$ DM) for the whole plant of faba bean varieties was 4.5, with significant differences between the varieties (Table 2.3). The highest N concentration was recorded in nodules ranging from 5.2 ± 0.3 (*Obse*) to 6.7 ± 0.4 (*Moti*) followed by roots ranging from 2.2 ± 0.2 (*Gebelcho*) to 3.0 ± 0.2 (*Degaga*), with significant differences between varieties. No significant differences were observed between the varieties for the shoot N concentration (Table 2.3).

The average whole plant total N content of the six faba bean varieties at flowering was 65.3 mg N plant$^{-1}$ without adjusting for the seed N contribution. When adjusting for seed N, the average plant N content was 37.3 mg N plant$^{-1}$ with significant differences between varieties (Table 2.3). The unadjusted whole plant total N content varied from 35.8 ± 4.6 (*Gebelcho*) to 78.3 ± 6.3 mg N plant$^{-1}$(*Obse*) and from 15.5 ±4.6 (*Gebelcho*) to 52.0 ± 9.8 mg plant$^{-1}$(*CS-20DK*) for the adjusted whole plant N content. Total N content of the shoot, root and nodule components was also significantly different for the varieties (Table 2.3). The proportion of N in the whole plant that is derived from seed N, from which the plants were grown, represented on average 48.2% and ranged between 34% (for *CS-20DK*) to 61% (for *Gebelcho*). The $\delta^{15}$N of the seeds at sowing was between -0.5‰ (for *CS-20DK*) to -1.1‰ (for *Walki*).

### 2.3.3 *B-value*

The $\delta^{15}$N of shoots, roots and nodules were significantly different between the varieties (Table 2.4). All the varieties exhibited a positive $\delta^{15}$N value for the nodules (in the range of +3.6 ± 0.9‰ for *Degaga* to +6.3 ± 0.6‰ for variety *Moti*) and a negative value for the shoots (ranging from -1.1 ± 0.3‰ in shoots of *CS-20DK* to -0.2 ± 0.2‰ in shoots of *Moti*). No significant differences were observed for the uncorrected whole plant $\delta^{15}$N value and the average of the varieties was -0.5‰. The corrected values of $\delta^{15}$N for the whole plant assuming Ps = 1 were significantly different between the varieties and varied from +0.5 ± 0.4‰ (*Moti*) to -1.9 ± 1.4‰ (*Degaga*).

### 2.3.4 %Ndfa, BNF and N Balance

The %Ndfa was significantly ($P < 0.05$) different between the varieties when using *B-values* corrected for seed N (corrected B) (Fig 2.2a). Variety *Moti* had the highest (84 ± 4.5%) %Ndfa while the lowest %Ndfa values were observed for *Degaga* (59 ± 4.2%). Applying the *B-value* uncorrected for seed N (uncorrected B) and average *B-value* from literature (average B) did not result in significant differences in %Ndfa for faba bean varieties. %Ndfa values ranged from 66.2 – 78.8% and 63.3 – 77.3% for uncorrected and average *B-values*, respectively. Further, BNF was significantly ($P < 0.05$) different for the varieties when estimated for two scenarios (corrected and uncorrected *B-values*). *Moti* showed the highest BNF (362 ±34.7 and 331 ± 33.6 kg N $ha^{-1}$) and *CS-20DK* the lowest (227 ± 29.3 and 218 ± 26.2 kg N $ha^{-1}$), respectively, for both scenarios (Fig 2.2b). However, the soil N balance did not differ significantly ($P > 0.05$) between varieties when quantified for the three scenarios (Fig 2.2c).

Furthermore, it was also shown that calculation of *B-value* without correcting for seed N or using an average *B-value* from the literature resulted in a biased %Ndfa, BNF and soil N balance estimation, ranging from an overestimation of +15% for *Degaga* to an underestimation of -19% for *Moti* (Fig 2.2a-c).

Table 2.3. N concentration (mg kg-1 DM) (mean ± SE) and total N content (mg N plant-1) (mean ±SE) of shoot, root, nodule and the whole plant of six faba bean varieties grown in N free medium. The N measurement is based on the dry yield of plant parts harvested at flowering (47 days after sowing). Means followed by different letters in a column are significantly different at $P < 0.05$. Whole plant (adjusted) refers to the total N content of whole plant minus seed N at sowing.

| | -------N Concentration (mg N $kg^{-1}$ DM)------ | | | | -------------------------Total N content (mg N $plant^{-1}$)-------------------- | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variety | Shoot | Root | Nodules | Whole plant | Shoot | Root | Nodules | Whole plant | Whole plant (adjusted) |
| *CS-20DK* | 2.7±0.3a | 2.9±0.3a | 6.5±0.2ab | 4.5 ±0.3ab | 52.3±9.1a | 18.9±1.9a | 3.6±0.6ab | 74.8±9.9a | 52.0±9.9a |
| *Degaga* | 2.9±0.2a | 3.0±0.2a | 5.7±0.4bc | 4.9 ±0.2a | 47.2±7.7a | 15.3±1.8ab | 4.5±0.9a | 66.9±9.5a | 40.4±9.5ab |
| *Gebelcho* | 2.3±0.4a | 2.2±0.2b | 5.6±0.1c | 3.9 ±0.4b | 24.2±4.7b | 10.2±1.4b | 1.3±0.2b | 35.8±4.6b | 15.5±4.6b |
| *Moti* | 2.3±0.4a | 2.5±0.2ab | 6.7±0.4a | 4.4 ±0.4ab | 48.2±10.2a | 19.5±2.1a | 5.0±1.5a | 72.8±12.4a | 46.1±12.4a |
| *Obse* | 2.8±0.3a | 2.9±0.1a | 5.2±0.3c | 4.9 ±0.2a | 53.5±5.6a | 20.3±1.0a | 4.5±0.9a | 78.3±6.3a | 39.1±6.3ab |
| *Walki* | 2.2±0.2a | 2.5±0.2ab | 5.5±0.2c | 4.1 ±0.3ab | 41.7±6.7ab | 16.5±1.6a | 4.8±1.1a | 63.0±9.1a | 33.3±9.1ab |
| Average | 2.5 | 2.7 | 5.9 | 4.5 | 44.5 | 16.8 | 3.9 | 65.3 | 37.7 |
| $LSD_{0.05}$ | 0.8 | 0.6 | 0.8 | 0.9 | 22.6 | 5.1 | 2.9 | 27.3 | 27.3 |

Table 2.4. B-values (mean ± SE) of the shoot, root, nodules and the whole plant of six faba bean varieties grown in N free medium. Means followed by different letters in a column are significantly different at $P < 0.05$. Whole plant (corrected) refers to the *B-value* obtained for whole plant after correction for the initial seed N contribution using a mass balance approach.

| | B value - $\delta^{15}N$ (‰) | | | | |
|---|---|---|---|---|---|
| Variety | Shoot | Root | Nodules | Whole plant | Whole plant (corrected) |
| *CS-20DK* | -1.1±0.3b | +0.4±0.4a | +6.3±0.6a | -0.5±0.3a | -0.3±0.6ab |
| *Degaga* | -0.7±0.2ab | -0.5±0.2bc | +3.6±0.9b | -0.5±0.1a | -1.9±1.5b |
| *Gebelcho* | -0.9±0.2ab | -0.6±0.1bc | +4.2±0.1ab | -0.9±0.1a | -0.6±0.4ab |
| *Moti* | -0.2±0.2a | -0.1±0.2ab | +6.3±0.6a | -0.04±0.2a | +0.5±0.4a |
| *Obse* | -0.9±0.3ab | -0.3±0.20bc | +4.6±1.1ab | -0.6±0.3a | -1.1±0.7ab |
| *Walki* | -0.7±0.2ab | -0.8±0.2c | +5.9±0.7a | -0.6±0.2a | -0.02±0.3ab |
| Average | -0.7 | -0.3 | +5.2 | -0.5 | -0.6 |
| LSD0.05 | 0.8 | 0.7 | 2.1 | 0.7 | 2.3 |

## 2.4 Discussion

It is often reported that dry matter production potential of a plant is the driving factor behind $N_2$ fixation when there is effective plant-rhizobium symbiosis (Unkovich and Pate, 2000). We determined dry matter yield (g plant$^{-1}$) of six faba bean varieties grown until full flowering in nutrient medium lacking N but inoculated with rhizobium. Significant differences were shown for the dry matter yield of the whole plant (1.6 ± 0.1 to 2.9 ± 0.2 g plant$^{-1}$) for the varieties. These differences in whole plant total dry matter yield could be attributed to the differences in dry matter yield produced by shoots, roots and nodules. The shoots represented 68 - 72% of the total dry matter of the whole plant whereas, the nodules and roots contributed for 1.3 - 3.8% and 23 - 30% to the total whole plant dry matter, respectively. The differences between varieties in terms of dry matter content of each plant part and the whole plant will therefore potentially influence the amount of atmospheric $N_2$ fixation between faba bean varieties. Dry matter yield is reported to be the driving factor behind $N_2$ fixation and that plant growth creates the demand for N (Unkovich and Pate, 2000).

The total N contributed by the seed at sowing to the whole plant N at flowering was in the range from 34% (*CS-20DK*) to 61% (*Gebelcho*), suggesting that seed size in faba bean is important for the accurate estimation of *B-values* and derived parameters such as BNF. The varieties used in the present study had average seed weight ranging from ca. 530 mg (*CS-20DK*) to 900 mg (*Obse*). The adjusted total N content for the whole plant was significantly different for the varieties ranging from 15.5 ± 4.6 mg N plant$^{-1}$ (*Gebelcho*) to 52.0 ± 9.8 mg N plant$^{-1}$ (*CS-20DK*). The adjusted values for total N content and $\delta^{15}N$ for the whole plant according to the mass balance model with Ps = 1 indicate that the total N and $^{15}N$ contributed by the seed at sowing to the total plant N was 48%, on average for the varieties, which was somewhat lower than the 54% reported by Lopez-Belido et al. (2010) for faba beans. This difference (48% vs. 54%) may be due to the differences in the type of varieties used, growing conditions and age of plants at harvest.

The$^{15}N$ abundance of $N_2$ fixing plants can vary with variety, growth stage and the plant part sampled (Boddey et al., 2000; Key-Boahen et al., 2002; Unkovich and Pate, 2000). In the present study, we analyzed the $^{15}N$ abundance of faba bean nodules, roots, shoots and whole plant at flowering stage. The $\delta^{15}N$ values were negative for roots, shoot and whole plant components but the strongly positive values for nodules showing significant isotopic discrimination against the heavier isotope during atmospheric $N_2$ fixation and during metabolism and N translocation in the plant system. The $^{15}N$ enrichment of the legume nodules is in agreement with Boddey et al. (2000), Okito et al. (2004) and Wanek and Ardnt (2002). This enrichment has been related to nodule metabolism (Wanek and Ardnt 2002). However, the fundamental isotope effects and discrimination processes leading to this non-uniform $^{15}$N discrimination within legumes has not been well described, despite a model developed by Robinson et al. (1998) for $^{15}$N signatures of nitrate grown plants. The $\delta^{15}$N values obtained in our study for nodules (+3.6 to +6.3‰), roots (-0.8 to +0.4‰) and shoots (-0.2 to -1.1‰) suggested wide deviations in $\delta^{15}$N signatures among plant parts and are similar to those provided by Boddey et al. (2000). $\delta^{15}$N values in the shoot (with mean *B-value* of -0.7‰) was similar to values reported by Unkovich et al. (2008) (-0.3‰ to -0.6‰) and Fan et al. (2006) (-0.7‰) but higher than values reported by Lopez-Bellido et al. (2010) (-1.7‰).

Regarding the $\delta^{15}$N of the whole plant, some authors (Nguluu et al., 2001) indicated that the values tend to be close to the $\delta^{15}$N of atmospheric N (0.0‰). However it cannot be 0.0‰ due to isotopic fractionation associated with biochemical processes in $N_2$ fixation (Shearer and Kohl, 1986). Lopez-Bellido et al. (2010) reported a mean value of -1.3‰ (uncorrected for seed N) and -1.5‰ (corrected for seed N) for the whole plant in faba beans. In our study, the uncorrected *B-value* for the whole plant of six faba bean varieties ranged from -0.04‰ to -0.9‰, while the corrected *B-value* ranged from +0.5‰ to -1.9‰. This shows a clear variety effect for the seed N corrected *B-value*. The findings of this study are in contrast to Lopez-Belido et al. (2010) who showed a non significant $^{15}$N discrimination pattern between the different faba bean plant parts with negative $\delta^{15}$N values for the nodules, root and shoot. Nevertheless, our results are in agreement with the reports of Boddey et al. (2000) and Okito et al. (2004) that showed significant isotopic discrimination between the nodules and the rest of plant parts. Our results further suggest that whole plant corrected *B-values* are the most correctly estimated *B-values* and therefore are more appropriate for the application of the $^{15}$N natural abundance technique to quantify BNF under field conditions.

Literature shows a wide range for %Ndfa and BNF by faba beans. Köpke and Nemecek (2010) reported %Ndfa up to 96% and BNF values ranging from 15 to 648 kg N ha$^{-1}$. Unkovich and Pate (2000) have shown %Ndfa of 20 to 97% and BNF of 12 - 330 kg N ha$^{-1}$ for faba beans. Peoples et al. (1997) in a survey of the on-farm contribution of BNF to soybean and mung bean in Pakistan and Nepal found physiologically incongruous values of over 100% for %Ndfa using a *B-value* determined for Australian soybean and mung bean varieties. This indicates that utilization of a *B-value* determined for one variety at one site or geographic region may not be valid for all situations even if dealing with the same legume species. The wide range of variation in %Ndfa and BNF may not only be a result of variations in growing conditions and varieties used, but also due to the methodology used for BNF determination including *B-values*. Peoples et al. (1997) and Unkovich et al. (1994) have indicated the likely magnitude of errors associated with the determination and application of *B-values* for estimates of $N_2$ fixation using the natural abundance technique. As a general rule, Unkovich et al. (1994) pointed out that the errors associated with an inaccurate *B-value* are likely to be high when %Ndfa estimates are greater than 85% and hence survey reports of Peoples et al. (1997) suggested that this is the case. The use of an average *B-value* from literature or a *B-value* that does not account for seed N contribution could also be a factor for the wide variation and incongruous estimates of %Ndfa and BNF in the literature.

In order to validate our estimated *B-values*, %Ndfa, BNF and soil N balance were calculated using field experiment data on $\delta^{15}N_{\text{reference plant}}$ (wheat), $\delta^{15}N_{\text{legume}}$ of the same six faba beans (Eq. 3) and three scenarios for *B-values* (corrected for seed N, uncorrected for seed N and *B-value* obtained from literature). The three *B-values* gave different results as depicted in figures 1a-c. We observed that overlooking seed N contribution during calculation of *B-value* or using an average *B-value* from the literature resulted in overestimation of %Ndfa, BNF and soil N balance by 15% for *Degaga* or underestimation by 19% for *Moti*. Moreover it masked the real difference and rank that exists between faba bean varieties. It is possible to note that %Ndfa of *Degaga*, *Moti* and *Walki* (68% each) overlapped with each other with same rank at the average *B-value* and that of *Degaga* and *Walki* (71% each) overlapped at the uncorrected *B-value* in the same manner. This overlap was unveiled however at the corrected *B-value* with clear difference and ranking of varieties, *Moti* being the top (Ndfa = 84%) and *Degaga* lower (Ndfa = 59%). A similar trend was noted also for BNF. This shows that *B-values* have differential influences on the estimated quantities of %Ndfa, BNF and soil N balance. This confirms our hypothesis that a single *B-value* taken from the literature or *B-value* determined without correcting for the initial seed N at sowing may result in inaccurate field estimates of BNF. To the best of our knowledge, we found only one published work in literature (Lopez-Bellido et al., 2010) that estimated *B-values* of faba beans taking into account the seed N contribution.

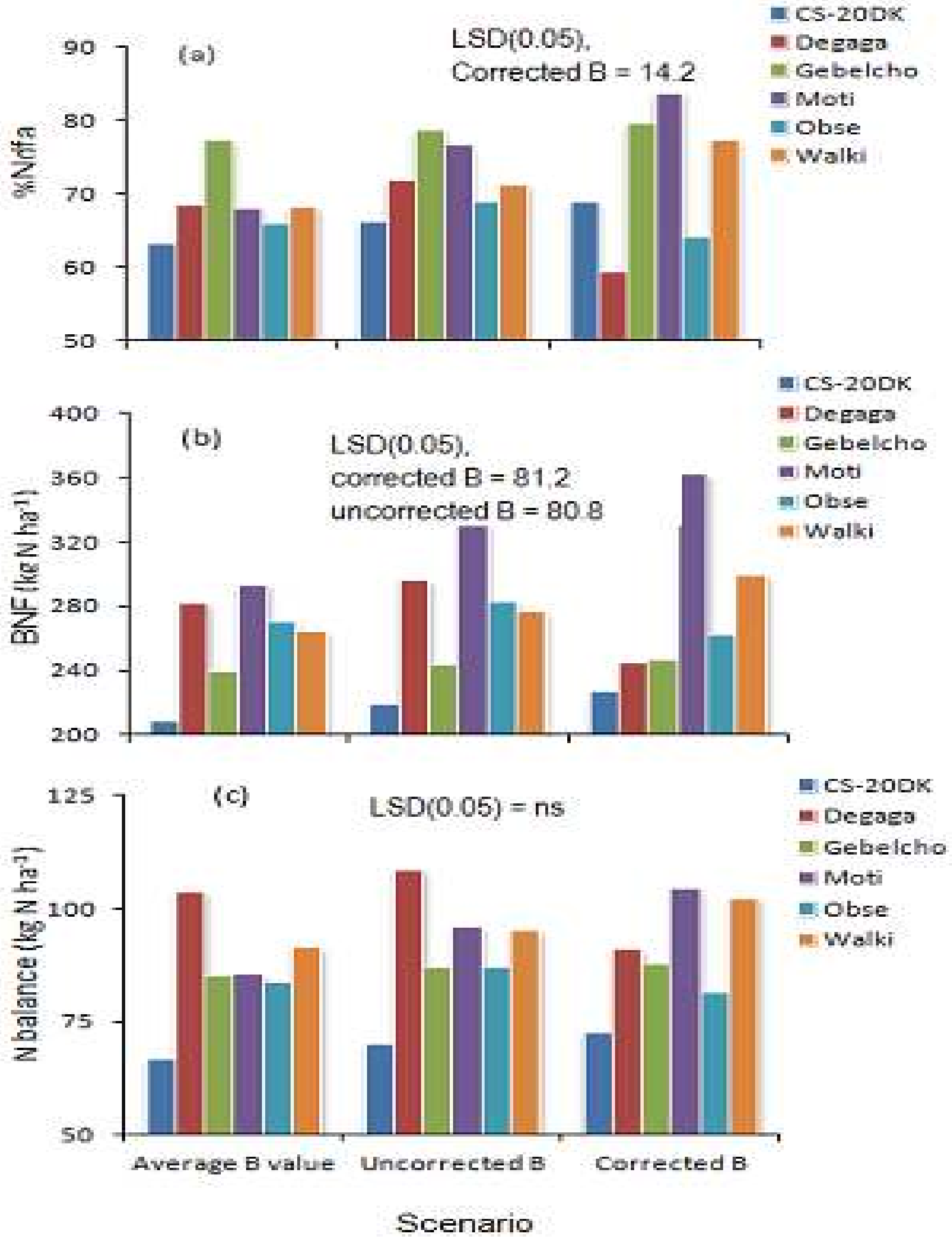

Figure 2.2. Effect of B value method on (a) %Ndfa, (b) BNF, and (c) soil N balance of six faba bean varieties grown on farmer's field at Dedo, Southwest Ethiopia estimated via $^{15}$N natural abundance using wheat as reference. Average B value is a B value of −0.85‰ obtained from literature, corrected B and uncorrected B are the observed whole plant B values corrected or not for the initial seed N, respectively. %Ndfa (a) of varieties was significantly different for Corrected B only ($LSD_{0.05}$=14.2; $P < 0.05$) and BNF (b) for both Corrected B ($LSD_{0.05}$=81.2; $P < 0.05$) and Uncorrected B ($LSD_{0.05}$=80.8; $P < 0.05$). N balance (c) of varieties was not significantly different for three of the scenarios ($P > 0.05$).

## 2.5 Conclusion

Quantifying biologically fixed N by legumes through the $^{15}N$ natural abundance techniques requires the determination of a so-called *B-value*. The *B-values* in our study differed significantly between varieties when the whole plant and seed N corrected value is used; which is the most correct and unbiased *B-value* approach. If the seed N corrected whole plant *B-values* are assessed against other approaches then it is noticed that BNF estimates could be biased by 34% (15% over- to 19% underestimation) when not accounting for varietal differences and seed-N. Moreover, real differences in varieties are masked, leading to erroneous rankings among varieties. This has especially consequences for correctly assessing N balances which is crucial for agronomic efficiencies. For this reason, the total N and $^{15}N$ content of large seeded legumes (e.g., faba beans) at sowing should have to be considered in the *B-value* determination to eliminate this error and minimize the distortion caused by the possible differences in N content of the seeds. Taking into account variety and seed N will result in more accurate %Ndfa and BNF under field conditions.

# Chapter 3

# Phosphorus utilization efficiency, biological nitrogen fixation and nitrogen balance of faba beans (*Vicia faba* L.)

Abstract

In most cropping systems of the African tropical highlands phosphorus (P) and nitrogen (N) fertilizer application is limited and maintaining sufficient available P and N for crop growth is a major challenge. Therefore the use of P efficient $N_2$-fixing legumes is a prerequisite for sustainable intensification of these low input agro-ecosystems. A study was undertaken on farmer's fields in the tropical highlands of Ethiopia in order to assess agronomic performance, P acquisition efficiency (PAE), P utilization efficiency (PUE), biological N fixation (BNF) and N balance of six improved faba bean (*Vicia faba*) varieties without and with P application. Varieties showed significant variations in PUE, but P application had no significant effect on PUE. Variety *Moti* demonstrated highest PUE of 272 kg grain kg$^{-1}$ P, which was 1.6-fold higher than the lowest PUE. PUE was significantly and positively correlated with grain yield (r = 0.542) and negatively correlated with shoot PAE (r = -0.541), indicating that PUE is important for grain yield. Significant differences between the varieties were also demonstrated for percentage N derived from the atmosphere (60 to 84%) and BNF (225 to 338 kg N ha$^{-1}$). BNF was significantly correlated with grain yield (r = 0.742) and total biomass yield (r = 0.791). The N balance was positive and ranged between 72 and 104 kg N $ha^{-1}$ with no significant differences between varieties. This experiment demonstrated that variations in grain and biomass yield of faba beans were largely due to differences in PUE and BNF and not due to P acquisition. Therefore we argue that genetic resources of faba bean varieties showing optimal agronomic performance, high PUE and high BNF under P limited conditions should be better explored. Introduction of such varieties in a low-input cereal-based cropping system could improve both soil N availability and protein production and enhance P use efficiency.

Keywords: Faba bean, Biological N fixation, N balance, P utilization efficiency, P harvest index

## 3.1. Introduction

The growing population pressure and declining soil fertility are among the critical problems limiting crop yields in Sub-Saharan Africa (SSA) (Chianu et al., 2011). The call for sustainable intensification of agriculture in SSA has gained support in recent years, especially in densely populated areas where natural fallows are no longer an option (Vanlauwe et al., 2012). Owing to these problems and the need to produce diverse products from the ever decreasing per capita landholdings, there is an urgent need to sustainably build up the small-land holder cropping systems of SSA (Chianu et al., 2011; Vanlauwe et al., 2012). However, most soils in SSA have deficiencies of available phosphorus (P) and nitrogen (N), which are the main constraints for cereal and legume production (Naab et al., 2009; Belane and Dakora, 2010). The use of chemical fertilizers could be a means to alleviate low nutrient levels and improve crop yields. However, at recommended application rates, chemical fertilizers are generally inaccessible to resource-poor farmers in SSA. A complementary strategy to increase soil fertility is the inclusion of $N_2$-fixing and P efficient grain legumes in traditional cropping systems (Belane and Dakora, 2010). $N_2$ fixing and P efficient grain legumes are a necessary component that complements fertilizer in SSA and should be introduced into crop rotation, e.g. legume-cereal rotations. Hence, fertilizer use efficiency in the cropping systems could be improved.

Among legumes, faba bean (*Vicia faba* L.) also called broad bean or horse bean is of great importance in legume-cereal production systems where it is used as break crop for cereals (Amanuel et al., 2000) and has the potential to enhance N and P nutrition of cereals (Habtemichial et al., 2007; Nuruzzaman et al., 2005; Rose et al., 2010). Faba bean, native to the Near East and Mediterranean basin (Zohary and Hopf, 2000), grows with optimal performance in good-structured clay or loam soils at temperatures between 18 and 27°C (Jensen et al*.,* 2010; Link et al., 2010). In Ethiopia faba bean is grown from 1300 to 3800 m altitude, but mostly at 2000 to 2500 m (Agegnehu and Chilot, 2009). The crop is well adapted to diverse soil types of Ethiopia where legumes are prominently used as traditional soil fertility maintenance crops in mixed cropping systems.

Soil P deficiency is a major constraint to increase (legume) crop yields in tropical and subtropical regions (Kirkby and Johnston, 2008). No or too little P fertilizer is actually used in those parts of the world and P input could have a major effect on food production (Syers et al., 2008). Low levels of soil available P and large crop responses to P fertilizer applications are common for both cereals and legumes (Gizaw et al., 1999; Ahmad et al., 2001; Agegnehu et al., 2006; Agegnehu and Chilot, 2009; Sanginga et al., 2000). P fertilizers are often expensive for the smallholder farmers due to the lack of locally available resources. However, if properly used at the recommended dose, time and mode of application P fertilizers can warrant intensification of smallholer cropping systems in SSA. Furthermore, fertilizer P can be fixed to Fe- and Al-oxides in tropical soils, which are unavailable pools for plants and hence restrict plant P utilization (Ahmad et al., 2001; Frageria and Barbosa Filho, 2008).

Selection of efficient P utilizing crops that yield well in soils with low available P is a cost-effective way of improving crop yields in low-input farming systems (Rose et al., 2011; Rose and Wissuwa, 2012). Such P-efficient crops would ideally have high P uptake (phosphorus acquisition efficiency, PAE) with very efficient use of P in biomass accumulation (phosphorus utilization efficiency, PUE) (Rose et al., 2011). PAE is defined as the ability of the plant to extract and take up the nutrient from the soil, and PUE as grain yield per unit of P taken up in aboveground plant material (Rose and Wissuwa, 2012). Thus, plant species producing equal grain yield at lower P uptake (compared to other plants) or higher grain yield at equal P concentrations would be considered to have better PUE.

Intra-species differences for PAE and PUE are well known for different grain legumes and cereals. Large genotypic differences in respect of PAE and PUE were reported for soybean (Furlani et al., 2002; Jemo et al., 2006), cowpea (Nwoke et al., 2007; Sanginga et al., 2000), common bean (Vadezl et al., 1999), faba bean (Stelling et al., 1996; Daoui et al., 2012) and wheat (Korkmaz et al., 2009; Manske et al., 2001, 2002; Sepehra et al., 1999). Therefore, one of the strategies to improve P nutrition of crops is through selection of P efficient varieties.

Moreover, there is evidence that legume genotypes may have developed various strategies to take up P from sparingly available sources (Marschner, 1998; Bagayoko et al., 2000; Nuruzzaman et al., 2005; Rose et al., 2010). However, since P efficient varieties strongly mine the soil P pool, they may not be considered as sustainable options for P management in cropping systems because soil P stocks are limited.

Atmospheric $N_2$ fixed by leguminous plants provides a sustainable source of N for agricultural intensification in SSA provided that the extra N fixed by the legume that remains in the soil is taken up efficiently by a subsequent crop following legumes. Among grain legumes, faba bean is one of the best $N_2$ fixers. The global annual estimate of biological N fixation (BNF) by faba bean has been estimated at 16.4 million Mg N year$^{-1}$ (Herridge et al., 2008). Under temperate conditions, Maidl et al. (1996) recorded $N_2$ fixation for faba bean in the range of 165–240 kg N ha$^{-1}$ resulting in a positive N balance of 84 kg N ha$^{-1}$ for the agro-ecosystem when only grain was removed. Jensen (1986) also reported a BNF of 186 kg N ha$^{-1}$ for faba bean with a corresponding percentage of N derived from the air (%Ndfa) of 66%. Amanuel et al. (2000) reported faba bean BNF from 169 - 210 kg ha$^{-1}$ with positive soil N balance of up to 92 kg N ha$^{-1}$ on Nitisols of Ethiopia. Moreover, Lopez-Bellido et al. (2006) obtained BNF by faba bean in the range between 31 and 144 kg ha$^{-1}$ with positive soil N balance up to 40 kg N ha$^{-1}$ in a Mediterranean environment. Research reports further indicate that substantial grain yield increments have been recorded for wheat following faba beans (Amanuel et al., 1991; Lopez-bellido et al., 2006; Habtemichial et al., 2007).

The large positive N balances and therefore positive carryover effects on subsequent cereal crop indicate that BNF by faba bean is very beneficial in a legume-cereal rotation. However, little effort has been made to select faba bean varieties for high BNF and PUE and positive soil N and P balances at low levels of available P in humid highland cropping systems. Better understanding and knowledge about differences among faba bean varieties is necessary to identify varieties suitable for humid tropical highland agro-ecosystems where fertilizers are not readily available. The general aim of this research was therefore to assess faba bean varieties that are productive under low soil P conditions or possess better capacities to take up and use P sources more efficiently. Identification and use of such varieties in the prevailing cropping systems could improve P nutrition of the legume and possibly also the non-legume components in a rotation (Abaidoo et al., 2007). The specific objectives were to determine (i) the agronomic performance, (ii) P utilization efficiency and (iii) BNF and N balance of elite faba bean varieties for humid highland cropping systems. These data allow us to propose and introduce best bet varieties for the tropical highlands taking into account the local context of highland pressure, abandoned fallowing and limited access to mineral fertilizers.

## 3.2. Materials and methods

### 3.2.1 Field sites

The study was conducted on farmer's fields in Dedo, located in southwestern Ethiopia at 7°28'48" N and 36°52'19"E and at an elevation of 2,160 m above sea level. Geologically, the area is associated with *Jimma Volcanics* with abundant rhyolites and trachybasalt (Regassa, 2009). The mean annual temperature is 20 ± 0.2 °C and average yearly rainfall is ca. 1880 ± 78.5 mm (data from 1975-2010, obtained from the National Meteorology Agency of Ethiopia). Total rainfall during the experimental year (2009/10) was 1562 mm and the minimum and maximum daily temperatures were 12 $^{o}$C and 23 $^{o}$C, respectively. Soils were classified as *Nitisols* in the FAO/WRB system. Participatory rural appraisal (PRA) research indicated that the cropping system is characterized by cereal (wheat, teff, barley) and legume (faba bean, field peas) production.

Cereal-legume based cropping system can be found on hill slopes and valley bottoms with good vegetation cover with trees, shrubs and Enset (*Enset ventricosum*). However, most agricultural land located on the hill slopes are affected by soil erosion and landslides, which has developed into one of the major crop production constraints particularly in the outfields. Low productivity, poor response of crops to chemical fertilizers and the very high cost of chemical fertilizers have also been reported as constraints (Bekele et al., 2010).

In the 2009-2010 growing season six faba bean genotypes (Table 3.1) were tested for agronomic performance, BNF and P use efficiency. The six varieties were selected because they had good adaptation potential, effective nodulation with local rhizobia and good agronomic performance among 15 accessions screened in the 2007-2008 cropping season at two sites (low and high altitude in the Gilgelgibe catchment) (Nebiyu et al., 2010). The six faba bean genotypes were grown with two levels of P fertilization (0 and 30 kg P ha$^{-1}$) given as Triple Super Phosphate (TSP) during the main cropping season (July - November) arranged factorial in a randomized complete block design with 3 replications. The TSP fertilizer was applied in rows and incorporated into the soil just before sowing. Seeds were obtained from the breeding center for faba beans at Holeta Agricultural Research center of the Ethiopian Institute of Agricultural Research. The varieties were not inoculated with rhizobia because they have proved to nodulate effectively without inoculation in the previous year. The plot size used was 4 m × 1.6 m. There were four rows in each plot and a planting density of 40 cm between rows and 10 cm within rows was maintained. Wheat was used as a reference crop to quantify BNF of faba bean. BNF was determined using the $^{15}$N natural abundance method (Unkovich and Pate, 2000; Peoples et al., 2009). All the necessary agronomic practices were applied during the growing season.

Table 3.1. *Source of varieties and some agronomic characteristics of six selected faba bean varieties

| Variety Name | Pedigree name | Source | Days to flowering | Days to maturity | Grain yield potential (Mg ha$^{-1}$) | Adaptation zone ($^{b}$m a.s.l.) |
|---|---|---|---|---|---|---|
| *CS-20DK* | CS20DK | Ethiopia | 57-67 | 145-160 | 1.5-3.0 | 2300-3000 |
| *Degaga* | R878-3 | ICARDA[a] | 45-62 | 116-135 | 2.0-4.5 | 1800-3000 |
| *Moti* | ILB4432 x Kuse 2-27-33 | ICARDA | 43-65 | 108-165 | 2.3-3.5 | 1800-3000 |
| *Gebelcho* | Tesfa x ILB4726 | ICARDA | 51-69 | 103-167 | 2.0-3.0 | 1800-3000 |
| *Obse* | CS20DK x ILB4427 | ICARDA | 43-65 | 87-166 | 2.1-3.5 | 1800-3000 |
| *Walki* | Bulga-70 x ILB4615 | Ethiopia/ICARDA | 49-61 | 133-146 | 2.0-4.2 | 1800-2800 |

[a] = *International Center for Agricultural Research in the Dry Areas, Aleppo, Syria*

[b] = meters above sea level

* data from MoARD (2009)

### 3.2.2 Soil sampling and analyses

Soil samples (0 - 30 cm) were collected from three different places within each replication and mixed to a representative composite sample per replication (nine sub-samples were composited). After air-drying, soil was ground and sieved (2 mm) and analyzed for pH, texture (Day, 1965), CEC and exchangeable bases (van Reeuwijk 2002), available P (Bray and Kurtz, 1945) and total phosphorus (Bowman, 1988). Total carbon (TC) and nitrogen (TN) were analyzed with an Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). Table 3.2 shows the soil physicochemical characteristics of the experimental site.

### 3.2.3 Plant sampling and analyses

During late flowering, five faba bean plants were randomly selected and uprooted from the central two rows and plant parts separated into root and shoot components. Soil adhering to the roots was removed by washing with tap water. The nodules from each plant were removed, counted and the dry weight was measured. The plots were additionally sampled for yield measurement at physiological maturity and hence the central two rows in each plot were harvested and subsequently separated into grains and crop residues (shoots and empty pods). Samples were dried at 70 °C for 48 hours and milled. Grain dry yield (GDY Mg ha$^{-1}$), total biomass yield (TBY Mg ha$^{-1}$ = GDY + shoot (SDY Mg ha$^{-1}$), empty pod (PDY Mg ha$^{-1}$) dry yield) and harvest index (HI% = GDY/TBY × 100) were calculated on dry weight basis.

### 3.2.4 Phosphorus analysis

P concentration (%) in shoot (PCs), grain (PCg), and pod (PCp) samples was determined according Chapman and Pratt (1961) using slight modifications according to Ryan et al. (2001). Plant samples (0.5 – 1.0 g) were ashed in porcelain crucibles for 5 hours at 550°C. The ash was dissolved in 5 mL 2N HCl and milli-Q water was added until a volume of 25 mL. The solution was filtered through Whatman No. 5 filters. P in filtrates was analyzed with an auto-analyser (Autoanalyzer 3, Bran+Luebbe, Norderstedt, Germany).

Table 3.2. Mean ± standard deviation of physicochemical soil characteristics of the farmer’s field in Dedo

| $pH_{KCl}$ | $pH_{H2O}$ | TC | TN | TP | Available P | Clay | Silt | Sand | CEC | Ca | Mg | Na | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (%) | (%) | -------(mg P $kg^{-1}$)------ | | ------------- (%) --------- | | | ---------------------($cmol_c$ $kg^{-1}$)----------------- | | | | |
| 5.1 | 5.6 | 2.8 | 0.2 | 1190 | 9.9 | 54.1 | 43.7 | 2.1 | 37.3 | 13.8 | 2.6 | 0.07 | 1.2 |
| ±0.16 | ± 0.15 | ± 0.33 | ± 0.03 | ± 96 | ± 3.80 | ± 2.09 | ± 2.11 | ± 0.33 | ± 1.79 | ± 1.31 | ± 0.29 | ± 0.01 | ± 0.28 |

TC = Total C, TN = total N, TP = total P

The following variables were then calculated: total P uptake (phosphorus acquisition efficiency, PAE, kg P $ha^{-1}$) in shoots (shoot PAE = %PCs × SDY t $ha^{-1}/10^3$), grains (grain PAE = %PCg × GDY t $ha^{-1}/10^3$), pods (pod PAE = %PCp × PDY t $ha^{-1}/10^3$), total above-ground biomass (total PAE = shoot PAE + grain PAE + pod PAE) and P harvest index in % (PHI = grain PAE/total PAE × 100). P utilization efficiency (PUE, kg grain $kg^{-1}$ P) was defined as the grain yield (GDY) per unit of P accumulated in the total aboveground biomass (Rose and Wissuwa, 2012) and is given as: GDY kg $ha^{-1}$/total PAE kg $ha^{-1}$.

Apparent P fertilizer recovery (APFR) was calculated as follows: APFR (%) = (total $PAE_{+P}$ - total $PAE_{-P}$)/P applied × 100, where total $PAE_{+P}$ and total $PAE_{-P}$ are total plant above-ground biomass P acquisition efficiency with and without P fertilization, respectively (Johnston and Syers, 2009).

### 3.2.5 Nitrogen analysis and BNF calculation

Total nitrogen content (TN) and $\delta^{15}N$ in plant samples was analyzed with Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). After determination of TN in the plant samples, the nitrogen yield was calculated as: Total N yield (kg N $ha^{-1}$) = dry mater yield (kg $ha^{-1}$) × %N/100.

The percentage of plant-N derived from atmospheric $N_2$ (%Ndfa), was calculated based on $^{15}N$ measurements in faba beans and reference plant (Peoples et al., 2009) as:

$\% \text{ Ndfa} = 100 \times (\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{legume}}) / (\delta^{15}N_{\text{reference plant}} - B \text{ value})$

Where, $\delta^{15}N_{\text{reference plant}}$ and $\delta^{15}N_{\text{legume}}$ are the whole plant $\delta^{15}N$ values of wheat and faba bean, respectively. Whole plant $\delta^{15}N$ values of faba beans were calculated as a weighted average from nodules, roots, shoots, pods and grains. The *B* value is the $\delta^{15}N$ value of faba beans grown in sterilized vermiculite for 47 days (flowering stage) with a nutrient solution free of N in a growth chamber (Vincent, 1970). Owing to its large seed size and high N content, the seeds sown to determine *B* value were also analyzed for total N and $\delta^{15}N$ to account for $^{15}N$ contribution by seeds. The *B* value was determined for each faba bean variety (Nebiyu et al., 2013). The amount of $N_2$ fixed by the legume (BNF, kg N $ha^{-1}$) = total N yield × (%Ndfa)/100. A simple input – output model was used to calculate the N balance. The input was total BNF and seed N while the output was all N removed via harvested products. Given the high seed N content and seed weight (Table 2.1), seed N was also taken as N input. The N balance (kg N $ha^{-1}$) was therefore calculated as as whole plant BNF plus seed N minus N exported via grain N.

### 3.2.6 Statistical analysis

A two-way analysis of variance for the factors variety and P level was performed using the general linear model (GLM) procedure of Statistical Analysis System (SAS) software version 9.2 (SAS, 2008) for all parameters except for APFR to which one-way ANOVA was performed. All the data were first checked for normality using the Proc univariate, option Normal procedure of SAS. Quantile-Quantile plot (Q-Q plot) on the residuals from ANOVA was taken into consideration for assessing the normality of the data. Due to lack of significant variety by P interaction ($P > 0.05$) for most of the response variables studied, except for PHI, the main effect of the factors are studied and discussed. For PHI, the variety by P interaction effect is presented in this thesis. Whenever the F-test denotes significant effect due to the main effect or interactions, the least significant difference (LSD) was used to compare treatment means at $P < 0.05$. In a balanced design where the same number of observations contributed to each mean, the LSD is more appropriate for mean comparison (Webster, 2007).

## 3.3. Results

### 3.3.1 Grain and total biomass yield, P response and P recovery of faba beans

Analysis of variance (ANOVA) revealed highly significant effects of the variety ($P < 0.01$) and P application ($P < 0.01$) factors for GDY and TBY and non significant ($P > 0.05$) effects for harvest index (HI%) of faba beans (Table 3.3). There was no significant interaction effect between variety and P levels for GDY and TBY. Varieties *CS-20DK* and *Gebelcho* produced the lowest grain and total biomass yield compared to others (Table 3.4). Application of 30 kg P $ha^{-1}$ resulted in highly significant ($P < 0.01$) response to grain and total biomass yield, but not to harvest index, irrespective of the varieties (Table 3.4) indicating that most of the faba bean varieties are responsive to P application.

There was no significant effect of variety ($P > 0.05$) for apparent fertilizer P recovery (AFPR) of faba beans. AFPR ranged between 20% (*CS-20DK*) to 43% (*Obse*) with no significant difference between varieties (Fig 3.1).

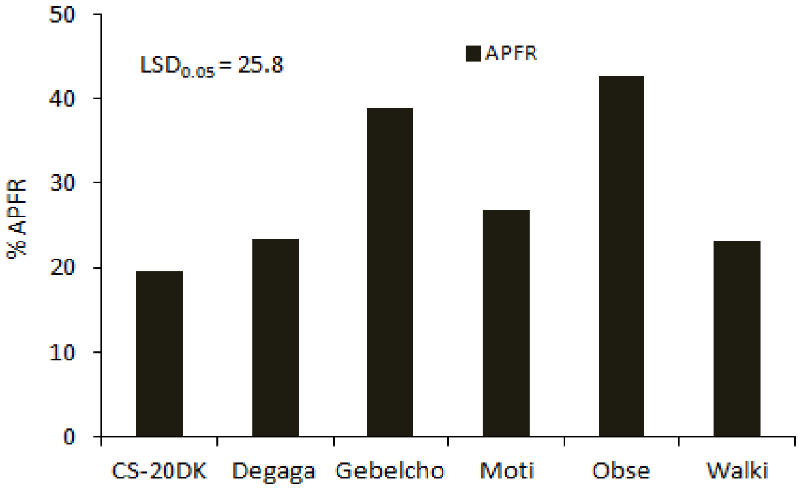

Figure 3.1. Apparent P fertilizer recovery (APFR) of six faba bean varieties grown on farmer’s field at Dedo, Southwest Ethiopia. Varieties did not differ significantly ($P = 0.351$) for APFR.

### 3.3.2. P concentration, P acquisition and utilization efficiency

ANOVA showed highly significant effects ($P < 0.01$) of the variety and P application on shoot and pod P concentrations. P grain concentration was affected only by the variety ($P < 0.05$) (Table 3.5). P shoot concentration was the highest for *CS-20DK*, *Gebelcho* and *Walki* and lowest for varieties *Obse*, *Degaga* and *Moti* (Table 3.5). *CS-20DK* contained significantly higher P concentration in its pods compared to others and *Walki* had the lowest P pod concentration (Table 3.5). Grain P concentration was significantly higher for *Gebelcho*, *Obse* and *Degaga* compared with others. P addition (30 kg ha$^{-1}$) significantly enhanced P concentration in shoots and pods but had no significant effect on grain P concentration irrespective of the varieties (Table 3.5).

Significant variations ($P < 0.05$) were observed in shoot and grain PAE for variety and P application. The effect of the variety was not significant for pod and total PAE, but P application did show significant effects on pod and total PAE (Table 3.3). *Gebelcho* and *Walki* demonstrated significantly highest shoot PAE of 6.5 kg P ha$^{-1}$ each followed by *CS-20DK* (Table 3.5). *Obse* did show the lowest shoot PAE (4.3 kg P ha$^{-1}$) but found to have the highest (17.0 kg P ha$^{-1}$) grain PAE which however was not statistically different from *Degaga*, *Moti* and *Walki*. Grain PAE was the lowest (11.3 kg P ha$^{-1}$) for *CS-20DK*. Total plant PAE ranged from 19.1 kg P ha$^{-1}$ for *CS-20DK* to 22.8 kg P ha$^{-1}$ for *Obse* with no statistical difference between varieties. P addition (30 kg ha$^{-1}$) significantly improved shoot (60%), pod (60%), grain (47%) and total plant PAE (55%) of faba beans irrespective of the variety. The PAE of pod, grain and total plant were significantly positively correlated with GDY and TBY (Table 3.6).

Table 3.3. P-values for the analysis of variance of dry grain yield (DGY), total dry biomass yield (TBY), harvest index (HI), apparent fertilizer P recovery (AFPR), P concentration in shoot (PCs), grain (PCg), pod (PCp), P acquisition efficiency in shoot (shoot PAE), pod (pod PAE), grain (grain PAE) and total plant (total PAE) and P utilization efficiency (PUE) and P harvest index (PHI) of six faba bean varieties and two P application levels. Factors with P-values less than 0.05 (P < 0.05) were considered to have significant effects on the respective response variable.

| Factor | GDY | TBY | HI | AFPR | PCs | PCp | PCg | Shoot PAE | Pod PAE | Grain PAE | Total PAE | PUE | PHI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety (V) | 0.007 | 0.0005 | 0.098 | 0.351 | 0.0001 | 0.0001 | 0.017 | 0.031 | 0.602 | 0.042 | 0.620 | 0.019 | <0.0001 |
| P application (P) | 0.0004 | 0.0001 | 0.103 | - | 0.0003 | 0.009 | 0.277 | <0.0001 | 0.002 | <0.0001 | <0.0001 | 0.191 | 0.241 |
| V*P | 0.238 | 0.362 | 0.195 | - | 0.446 | 0.359 | 0.342 | 0.629 | 0.597 | 0.390 | 0.611 | 0.133 | 0.042 |
| CV (%) | 21.9 | 13.7 | 10.2 | 49 | 17.5 | 21.7 | 13.7 | 24.3 | 43.7 | 22.1 | 19.2 | 22.4 | 7.2 |

CV = coefficient of variation

Table 3.4. Main effect of varity and P-application on dry grain yield (DGY t ha$^{-1}$), total above ground biomass yield (TBY t ha$^{-1}$) and harvest index (HI%) of six faba bean varieties. The values are mean ± SE. Means followed with different letters in a column are significantly different at P<0.05.

| Variety | GDY (t ha$^{-1}$) | TBY (t ha$^{-1}$) | HI (%) |
|---|---|---|---|
| *CS-20DK* | 3.7 ± 0.3bc | 7.6 ± 0.5b | 49.0 ± 2.0 |
| *Degaga* | 4.8 ± 0.4ab | 9.9 ± 0.6a | 48.4 ± 1.9 |
| *Gebelcho* | 3.2 ± 0.5c | 7.5 ± 0.8b | 42.3 ± 1.7 |
| *Moti* | 5.5 ± 0.6a | 10.8 ± 0.6a | 50.5 ± 2.9 |
| *Obse* | 4.7 ± 0.7ab | 10.0 ± 1.1a | 45.9 ± 2.6 |
| *Walki* | 4.5 ± 0.4ab | 9.6 ± 0.7a | 47.0 ± 1.3 |
| $LSD_{0.05}$ | 1.2 | 1.5 | Ns |
| P application (kg ha$^{-1}$) | | | |
| 0 | 3.7 ± 0.3B | 8.0 ± 0.5B | 45.8 ± 1.4A |
| 30 | 5.1± 0.3A | 10.4 ± 0.4A | 45.5 ± 1.1A |
| $LSD_{0.05}$ | 0.7 | 0.9 | 3.3 |

PUE was significantly affected by variety, but P application had no significant effect (Table 3.3). Varieties *Moti* and *Degaga* demonstrated highest PUE of 272.3 and 230.7 kg grain kg$^{-1}$ P, respectively with no statistical difference between these varieties (Table 3.5). *Gebelcho* showed lowest PUE of 164.7 kg grain kg$^{-1}$ P. Although not significant, application of 30 kg P ha$^{-1}$ reduced average PUE of faba bean plants from 224.4 kg grain kg$^{-1}$ P (0 P) to 202.9 kg grain kg$^{-1}$ P (30 P) (Table 3.5). PUE was significantly positively correlated with GDY (r = 0.542; P <0.05) and significantly negatively correlated with P concentrations in shoot (r = -0.626; P <0.05), grain (r = -0.726; P <0.01) and shoot PAE (r = -0.541; P <0.05) (Table 3.7).

Table 3.5. Main effect of varierty and P-application on P concentration (mg P $kg^{-1}$ DM), P-acquisition efficiency (PAE kg P $ha^{-1}$) of shoot, pod, grain and total plant, and P utilization efficiency (PUE kg grain $kg^{-1}$ P) of six faba bean varieties. All the measurements are based on the dry yield. The values are mean ± SE. Means followed with different letters in a column are significantly different at P<0.05.

| | P concentration<br>---------(mg P $kg^{-1}$ DM)--------- | | | PAE<br>----------------(kg P $ha^{-1}$)----------- | | | | PUE<br>(kg grain $kg^{-1}$ P) |
|---|---|---|---|---|---|---|---|---|
| Variety | Shoot | Pod | Grain | Shoot | Pod | Grain | Total | |
| *CS-20DK* | 0.23a | 0.13a | 0.31b | 6.3ab | 1.5a | 11.3c | 19.1a | 196.3bc |
| *Degaga* | 0.14b | 0.07cd | 0.32ab | 4.7cb | 1.2a | 15.4ab | 21.3a | 230.7ab |
| *Gebelcho* | 0.22a | 0.09b | 0.36a | 6.5a | 1.2a | 11.9bc | 19.7a | 164.7c |
| *Moti* | 0.15b | 0.08bc | 0.28b | 5.2abc | 1.5a | 14.9abc | 21.6a | 272.3a |
| *Obse* | 0.12b | 0.07bcd | 0.36a | 4.3c | 1.5a | 17.0a | 22.8a | 205.0bc |
| *Walki* | 0.20a | 0.06d | 0.31b | 6.5a | 1.0a | 14.0abc | 21.6a | 213.2bc |
| $LSD_{0.05}$ | 0.04 | 0.02 | 0.05 | 1.6 | 0.7 | 3.7 | 4.8 | 57.3 |
| P application (kg $ha^{-1}$) | | | | | | | | |
| 0 | 0.15B | 0.07B | 0.32A | 4.3B | 1.0B | 11.4B | 16.4B | 224.4A |
| 30 | 0.20A | 0.09A | 0.33A | 6.9A | 1.6A | 16.8A | 25.4A | 202.9A |
| $LSD_{0.05}$ | 0.02 | 0.01 | 0.03 | 0.9 | 0.4 | 2.1 | 2.8 | 33.1 |

The PHI (proportion of P exported via grains) significantly varied due to the interaction effect of varieties and P application levels (Table 3.7). Without P application, PHI ranged from 55.9 to 76.4%. This range was 59.9% to 74.4% for the 30 P. *CS-20DK* and *Gebelcho* had significantly lower PHI at both 0 P and 30 kg P. Varieties *Moti* and *Degaga* had the highest phosphorus harvest index (%) in order of 76.4 and 75.2% at 0 P and *Obse* 74.4 % at 30 P applications, though these varieties did not differ significantly from each other.

Table 3.6. Correlation coefficients between grain yield (GDY), total biomass yield (TBY), P utilization efficiency (PUE) versus the P acquisition efficiency (shoot PAE, grain PAE) and biologically fixed N (BNF) of faba beans.

| | Shoot PAE | Grain PAE | PUE | BNF |
|---|---|---|---|---|
| GDY | 0.163 $^{ns}$ | 0.818** | 0.542* | 0.742** |
| TBY | 0.228 $^{ns}$ | 0.918** | 0.125$^{ns}$ | 0.791** |
| PUE | -0.541* | 0.086 $^{ns}$ | 1.00 | -0.016$^{ns}$ |

* = correlation is significant at $P < 0.05$; ** = correlation is significant at $P <= 0.01$; ns = correlation is not significant

Table 3.7. Interaction effect of variety and P-aplication on P-harvest index (PHI%) of six faba bean varieties at two P application levels. The values are mean ± SE. Means followed with different letters in a column and row are significantly different at $P < 0.05$.

| Variety | PHI (%) | |
|---|---|---|
| | 0P | 30P |
| *CS-20DK* | 58.7 ± 1.5ed | 59.9 ± 2.6ed |
| *Degaga* | 75.2 ± 1.6a | 69.8 ± 0.9abc |
| *Gebelcho* | 55.9 ± 3.5e | 62.9 ± 1.8cde |
| *Moti* | 76.4 ± 2.6a | 64.2 ± 0.7cd |
| *Obse* | 73.4 ± 8.4ab | 74.4 ± 2.7a |
| *Walki* | 66.2 ± 1.6bcd | 63.1 ± 2.9cde |
| $LSD_{0.05}$ | 8.2 | |

### 3.3.3. Biological nitrogen fixation and N balance

Nodulation, as observed at the late flowering stage of faba bean, was effective for all the varieties. However, no variation ($P > 0.05$) was observed in nodule number and weight against variety or P application (Table 3.8). ANOVA further showed that the %Ndfa and the total N fixed were significantly affected by variety ($P < 0.05$) and P application ($P < 0.05$). The total N yield was however significantly affected ($P < 0.05$) both by variety and P application. There was no significant interaction effect between variety and P levels ($P > 0.05$) for %Ndfa, N yield and N fixed (Table 3.8). Table 3.9 shows mean values of nodule number and weight, %Ndfa, total N yield, N fixed and N balance for the variety and P application factors. Nodule number for the varieties ranged from 55 ± 12 to 118 ± 23 plant$^{-1}$ and nodule weigh from 68.6 ± 11.9 to 107.1 ± 34.5 mg plant$^{-1}$. %Ndfa ranged between 60% (*Degaga*) to 84% (*Walki*) for the factor variety. *Moti* and *Gebelcho* also had 78% and 75% Ndfa respectively, with no statistical difference from *Walki*. P addition significantly reduced %Ndfa from 76.6% (0 P) to 67.7% (30 P) on average. Total N yield ranged from 313 kg N ha$^{-1}$(*Gebelcho*) to 427.8 kg N ha$^{-1}$ (*Moti*) for the factor variety. *Degaga* (414 kg N ha$^{-1}$) and *Obse* (407 kg N ha$^{-1}$) showed statistically similar N yield values with *Moti*. At 30 P, N yield was significantly higher (431.6 N kg ha$^{-1}$) than the 0 P (332.2 kg N ha$^{-1}$). *Moti* fixed the highest total N (338.3 kg N ha$^{-1}$) followed by *Walki* (325.6 kg N ha$^{-1}$) and *Obse* (274.5 kg N ha$^{-1}$). Total N fixed did not significantly differ between 0 P and 30 P applications. The N balance of the different varieties was positive with no significant difference amongst the varieties and ranged from 72.5 kg N ha$^{-1}$ (*CS-20DK*) to 104.3 kg N ha$^{-1}$ (*Moti*). P addition did not bring a significant change in N balance. We found that variety *Moti* had highest amount of N fixed, highest PUE and was among the best grain and biomass yielding faba bean varieties.

Table 3.8. P-values for analysis of variance of percentage N derived from atmosphere (%Ndfa), N yield, N fixed and N balance of six faba bean varieties and two P application levels. Factors with P values less than 0.05 ($P < 0.05$) were considered to have significant effects on the respective response variable.

| Factor | Nodule number | Nodule weight | %Ndfa | N-yield | N-fixed | N-balance |
|---|---|---|---|---|---|---|
| Variety (V) | 0.201 | 0.884 | 0.023 | 0.04 | 0.034 | 0.470 |
| P application (P) | 0.533 | 0.523 | 0.332 | <0.0001 | 0.094 | 0.244 |
| V*P | 0.653 | 0.408 | 0.492 | 0.4005 | 0.797 | 0.309 |
| CV (%) | 16.3 | 17.1 | 16.2 | 13.7 | 24.9 | 34.6 |

CV = coefficient of variation

Table 3.9. Main effect of variety and P-application on nodule number, nodule weight, percentage N derived from atmosphere (%Ndfa), N yield, N fixed and N balance of six faba bean varieties. The values are mean ± SE. All the measurements are based on the dry yield. Means followed with different letters in a column are significantly different at P<0.05.

| Variety | Nodule number | Nodule weight (g plant$^{-1}$) | %Ndfa | N yield (kg N ha$^{-1}$) | N fixed (kg N ha$^{-1}$) | N balance (kg N ha$^{-1}$) |
|---|---|---|---|---|---|---|
| *CS-20DK* | 68.6±16.2a | 0.1±0.02a | 68.2±7.9bc | 336.4±26.1bc | 225.3±27.0c | 72.5±16.3a |
| *Degaga* | 82.5±18.8a | 0.1±0.01a | 60.3±3.4c | 414.5±29.0a | 248.1±17.1bc | 90.9±10.1a |
| *Gebelcho* | 61.0±19.2a | 0.1±0.03a | 75.1±4.8ab | 312.9±33.5c | 232.8±27.8c | 87.7±12.3a |
| *Moti* | 63.8±17.9a | 0.2±0.03a | 78.4±4.4ab | 427.8±26.6a | 338.3±34.4a | 104.3±17.8a |
| *Obse* | 118±23.4a | 0.1±0.02a | 66.9±3.1bc | 407.0±40.9a | 274.5±33.8abc | 80.8±7.3a |
| *Walki* | 55.0±11.9a | 0.1±0.03a | 83.9±4.8a | 392.5±25.7ab | 325.6±16.9ab | 102.4±11.6a |
| $LSD_{0.05}$ | 64.0 | 0.07 | 13.9 | 62.5 | 81.9 | 37.1 |
| P application (kg ha$^{-1}$) | | | | | | |
| 0 | 70.1±10.1A | 0.1±0.01A | 76.6±2.6A | 332.2±17.7B | 254.2±16.5A | 76.5±5.5A |
| 30 | 79.6±12.1A | 0.1±0.01A | 67.7±3.5B | 431.6±12.8A | 294.1±18.4A | 89.8±9.0A |
| $LSD_{0.05}$ | 31.2 | 0.04 | 8.1 | 36.1 | 47.3 | 21.4 |

## 3.4. Discussion

Our results showed that, there was a varietal difference in grain and total biomass yield in the Ethiopian faba bean genetic resources. The varieties *CS-20DK* and *Gebelcho* produced the lowest grain and total biomass yield but harvest index has not significantly changed with varieties or P application. Thus, the differences in yield could be related to changes in dry matter production with no change in partitioning indicating that harvest index in faba bean is conservative across varieties and P level. P application also resulted in a significant response to grain and biomass yield of faba beans suggesting how much grain and biomass yield was lost when no P was applied. Significant grain yield increase by P application in faba beans growing in P-limited soils was previously reported in Ethiopia (Agegnehu and Fessehaie, 2006; Amanuel et al., 2000) and Morocco (Daoui et al., 2012). Our results further support the findings of previous studies (Bolland et al., 2000; Daoui et al., 2012) indicating that phosphorus is the major nutrient element required for grain production of faba beans. Further, the differences in grain yield might have arisen from the significant differences in the PUE of genotypes such that PUE was significantly correlated with GDY ($r = 0.542$; $P < 0.05$). In view of this, the superior grain yield performance of variety *Moti* may further suggest that *Moti* may have mechanisms that efficiently translocate P in the internal plant system and avoid too much P storage in the tissue (had lower tissue P concentrations) such that growth is not limited by P shortage. Daoui et al. (2012) also reported a genotypic effect on grain and biomass production potential of faba beans that some genotypes do respond under various levels of P application and this corroborates our findings.

The shoot PAE and shoot P concentration of varieties *CS-20DK* and *Gebelcho* was the highest, however did not result in a corresponding high PUE and high grain and biomass yield. This also suggested that the difference in grain and biomass production among the varieties primarily stems from the PUE and not from the PAE or P concentration because varieties with the highest P concentration and PAE resulted in a lower grain and biomass yield; e.g. *CS-20DK* and *Gebelcho*. Correlation analysis also revealed negative association of PUE with P concentrations in shoot ($r = -0.626$; $P < 0.05$), grain ($r = -0.726$; $P < 0.01$) and shoot PAE ($r = -0.541$; $P < 0.05$). Likewise, grain and biomass yield were also negatively correlated with P concentrations in shoot, pod and grains. Baon et al. (1993) showed that PUE of plants usually decreases with increased shoot P concentration. Our results are in conformity with the findings of Stelling et al. (1996) and Daoui et al. (2012) who found that PUE was more important than PAE to explain genotypic variations in P use (in terms of grain and biomass production) by faba beans. However, Nuruzzaman et al. (2005) showed that high PUE of faba beans was related to PAE from low available P sources via the plant's extensive root system that can explore a larger volume of soil to access relatively immobile nutrients like phosphorus. If optimal performance in low P soils is one of the major selection criteria for faba beans, all the varieties, excepting *Moti*, that require P application for enhanced biomass production are not preferred. Most legume breeding programs in Africa look for genotypes that give high yield under low soil P situations. *Moti* meets this requirement and could fit well in cropping systems where little or no fertilizer is used and where crop yields are associated with subsistence farming.

Significant responses of faba bean varieties were also observed for grain and pod P concentrations and grain PAE. The grain P level was generally higher than the shoot and pod P levels in the present study. Rose et al. (2007) suggested that crop P requirement after flowering is largely dominated by two competing processes (P sinks): the P requirements of vegetative tissues to continue normal growth and development until senescence and the P demand of the developing grain. Further, Raboy (2009) showed that levels of P in grains are well above the P levels required for normal cellular function. The lower levels of P in faba bean pods in the present study may possibly imply that pods are not strong P sinks in the plant system. Therefore, it could be suggested that lower levels of P in pods would be due to reduced sink strength of the pods as P storage organs rather than a lower physiological P requirement by pods. Rose and Wissuwa (2012) had similar discussions on lower sink strength of grains. Reduced P sink strength of the pods, and certainly of grains, may therefore be an additional PUE parameter in further PUE studies involving grain legumes. Thus, under low P conditions grain yield is higher. Rose and Wissuwa (2012) also argue that targeting genotypes for low grain P can improve PUE of the system; however the potential impact of a low grain P characteristic on nutritional value and seedling growth and vigor in P limited soils needs further investigation.

Several studies have investigated the possibility of reducing grain P levels using criteria such as PHI at maturity (Batten, 1992; Jones et al., 1992; Manske et al., 2002; Rose et al., 2010). Our data also showed that there was significant interaction effect between variety and P on PHI. The total P exported in the grain of varieties *CS-20DK* and *Gebelcho* was lower compared to others at both P levels. The lower PHI and grain PAE of *CS-20DK* and *Gebelcho* may indicate that P was not always efficiently translocated from shoots into grains though these varieties demonstrated a relatively higher shoot PAE. This suggest that, due to their low P export to grains *CS-20DK, Gebelcho* and *Walki* may also be suitable varieties when aiming at faba bean genotypes with the potential of improving the P nutrition in crop rotations via its residues. Blair (1993) suggested that an ideal P efficient plant should have low PHI so as to retain more P in straw for subsequent use by less efficient crops in the rotation scheme.

The relatively high %Ndfa values for faba bean genotypes for both P treatments is in line with values reported previously for faba bean (Carranca et al., 1999; Amanuel et al., 2000; Killian et al., 2001; Lopez-Bellido et al., 2006) but was lower than the maximum reported (97%) by Schwenke et al. (1998). Literature shows a wide range for %Ndfa and BNF by faba beans. Köpke and Nemecek (2010) reported %Ndfa for faba beans up to 96% and BNF values ranging from 15 to 648 kg N ha$^{-1}$. This wide range of variation in %Ndfa and BNF may be a result of variations in faba bean growing conditions, varieties used, but also the methodology used for BNF determination including correct use of *B-values* and reference plants. The impact of the *B-value* has been discussed in several reviews (Peoples et al., 2002; Unkovich et al., 2008). Since we determined specific *B-values* for each variety, ranging from -1.9 ±1.5‰ for *Degaga* to +0.5 ±0.4‰ for *Moti*, the obtained results on BNF could be regarded as specific.

The total nitrogen fixation by faba bean varieties generally increased by P application and was most pronounced for variety *Moti*, which fixed about 338 kg N ha$^{-1}$. This value was higher than that reported by Beck et al. (1991), Schwenke et al. (1998) and Carranca et al. (1999). The increased amount of N fixed with P application is due to increased levels of biomass yield because %Ndfa slightly decreased with P application. Peoples et al. (2009) also reported that the mount of N fixed is generally controlled by faba bean growth rather than by %Ndfa implying that BNF in faba beans is largely controlled by variety. This also agrees with the report of Sanginga et al. (1991), which stated that the effect of P on $N_2$ fixation was mainly on the total amount of N fixed rather than on the %Ndfa. Further, Abdel-Wahab et al. (1994) reported a similar finding in Egypt and Amanuel et al. (2000) in Ethiopia whereby P application reduced the %Ndfa in faba beans due to an increased N derived from soil. The addition of 30 P might have enhanced the plant growth and development, including extensive root system proliferation, which could help the plant to explore a larger volume of soil to absorb more soil N, which may eventually result in a lower %Ndfa. Our observation that %Ndfa decreases when P fertilizer is added is at odds with other studies which found that adding P fertilizer to P-deficient legume plants increases the %Ndfa (e.g. Cadish et al., 1989; Cadish et al., 1993; Almeida et al., 2000). In our study, the relatively high levels of %Ndfa at 0 P may suggest that faba beans can maintain a high rate of $N_2$ fixation under low available P conditions, which also demonstrated the fact that under P limited soil conditions, effective nodulation and $N_2$ fixation can occur in faba beans. Nodules are strong P sinks but there was no significant effect ($P > 0.05$) of variety or P application on nodule number and weight per plant, and we observed effective (pink and dark pink colored nodules) and higher number of nodules per plant ranging from 55 for *Walki* to 118 for *Obse*. The total P pool in the study area was high and hence mycorrhyzal fungi might have helped the plants to extract and use this total soil P pool. Mycorrhyzal fungi are important for P acquisition, plant performance, better photosynthesis and higher efficiency on $N_2$ fixation (Jia et al., 2004). This fact may justify the lack of response on the applied P to nodule number, nodule weight and %Ndfa.

The contribution of the faba bean to soil N, as measured via the N balance, was positive for all varieties and both P application levels. Although not statistically different from other varieties, *Moti* and *Walki* showed the largest soil N surplus (104 kg N $ha^{-1}$ and 102 kg N $ha^{-1}$, respectively). Similarly, Zapata et al. (1987) and Amanuel et al. (2000) reported a positive soil N balance for faba bean after harvesting pods. The positive N-balance after harvesting of faba bean grains generates two advantages for subsistence farmers. First, the positive N balance could be obtained without P addition and second, incorporation of legume residues into the soil is beneficial for a subsequent non-leguminous crop that can be produced with a reduced level of inorganic fertilizer. This represents a big advantage for subsistence cereal-based production systems in SSA, where fertilizers are too expensive or unavailable. The N balance of *Moti* and *Walki* is higher than the optimum mineral N application rate (82 kg N $ha^{-1}$) recommended for wheat production on farmers' fields on Nitisols of Southeast Ethiopia (Amanuel et al., 1991).

In low-input systems of SSA, crop residues are frequently removed from the field to be used as animal feed or as a source of energy. In order to change these practices, farmers need to be convinced in that crop residues have more financial benefits when used as sources of plant nutrients (Bakht et al., 2009). The price of 1 kg urea fertilizer is on average ca. \$1.6 in SSA (Chianu et al. 2011). *Moti* returned about 104 kg N $ha^{-1}$ to the soil, which is equivalent to 226 kg urea (46% N) and thus gives an economic advantage of \$361 $ha^{-1}$ to the resource poor farmers. This represents a big advantage in subsistence production systems where fertilizer inputs are too expensive or unavailable. BNF by faba beans can thus contribute to increased yields of subsequent cereals and therefore improve soil fertility if residues are incorporated to the soil. However, the N use efficiency of organic N might not be as high as that of fertilizer N. So, the N balance of *Moti* and *Walki* might not be enough to reach the same cereal yield as with mineral fertilizers.

In general, we found that varieties that showed higher $N_2$ fixation (e.g. Moti) also gave the greatest grain and biomass yield, positive N balance and high PUE indicating that it is possible to concurrently select faba beans for both high $N_2$ fixation and N-balance and greater grain and biomass yield and high P utilization efficiency under low P conditions. Belane and Dakora (2010) have also demonstrated simultaneous selection of cowpeas for $N_2$ fixation and greater grain yield.

## 3.5. Conclusion

In conclusion, the data from this study carried out in P-deficient farmer's fields showed considerable differences in grain and biomass yield, BNF and P utilization efficiency among the six faba bean varieties that came out best in a previous screening for adaptation and agronomic performance using 15 varieties. It was moreover found that a difference in P use efficiency was largely due to differences in P utilization efficiency. There was much less difference in P uptake in low-P conditions, hence little difference in P acquisition efficiency. The best variety (*Moti*) yielded more than twice the quantity of grains than the worst variety (*Gebelcho*). The variety *Moti* showed highest P utilization efficiency, highest BNF, positive N balance and produced the largest amount of biomass and provided highest grain yield. It could therefore be an ideal variety for low P soils in the cool humid highland agro-ecosystems. However, it is also important to evaluate its effect on soil N and P status and the carryover effect of residue derived N and P nutrition of subsequent cereal crops. Further, assessing the N and P use efficiency of the crop residue in combination with low levels of mineral fertilizers in a subsequent cereal crop may warrant sustainable agricultural intensification for smallholders. Our results finally suggest the need to take P requirements of faba bean varieties into consideration when selecting and introducing new varieties for highland agro-ecosystems, and not only BNF; hence careful exploration of genetic resources is an essential component for sustainable intensification of African Agriculture.

# Chapter 4

# Nitrogen and phosphorus benefits from faba bean residues to subsequent wheat crop in the humid highlands of Ethiopia

After: *Amsalu Nebiyu, Adeline Vandorpe, Jan Diels and Pascal Boeckx. Nitrogen and phosphorus benefits from faba bean (Vicia faba L.) residues to subsequent wheat crop in the humid highlands of Ethiopia. Nutrient Cycling in Agroecosystems (under revision).*

## Abstract

Legume–cereal rotation is an important component of the cropping systems of smallholder farmers in the highland agro-ecological zones of Ethiopia. However, little is known about the rotational effects of faba beans on the performance of subsequently grown wheat. We therefore conducted a two phase field experiment to test to what extent high $N_2$-fixing and P efficient faba bean varieties could benefit subsequently grown wheat. In the first phase, three faba bean varieties (*Degaga*, *Moti*, *Obse*) were grown at four levels of P fertilization (0, 10, 20 and 30 kg P $ha^{-1}$) along with local faba bean and a reference wheat but without any fertilization. $N_2$-fixation, soil N balance and P uptake were determined for the faba beans. The N balance was determined via two possible residue management scenarios: Scenario-I assumed that all the aboveground biomass is exported from the fields and scenario-II assumed that all the above ground biomass except grains and empty pods is returned to the soil. In the second phase, the N and P benefits of faba beans to rotational wheat were assessed. In the first phase, average amounts of $N_2$-fixed significantly ranged 258 ± 16.8 to 387 ± 14.8 kg N $ha^{-1}$. Scenario-I gave a negative net N balance (kg N $ha^{-1}$) in the range of -78.4 ± 6.1 (*Degaga*) to -2.8 ± 8.7 (*Moti*) with significant differences between varieties. Scenario-II showed that all balances were significantly ($P < 0.01$) improved and the varieties were positively contributing N to the system in the range 58.7 ± 13.4 (*Degaga*) to 174.8 ± 13.7 (*Moti*) kg N $ha^{-1}$, which is equivalent to 126 – 380 kg N $ha^{-1}$ in the form of UREA (46% N). Variety *Moti* accumulated significantly higher P (14.4 ± 0.7 kg P $ha^{-1}$) in haulms compared to others. In the second crop phase, biomass and grain yield of wheat grown after the faba beans were improved significantly ($P < 0.05$) by 112 and 82%, respectively compared to the yield of wheat after wheat. P application to the preceding faba bean varieties also significantly improved the total biomass and grain yield of the succeeding wheat with significant linear relationship between P application to the previous faba beans and wheat biomass ($R^2 = 0.97$) and grain yield ($R^2 = 0.97$). Further, the highest grain N uptake (58.7 ± and 52.8 kg N $ha^{-1}$) was obtained at P application of 30 and 20 kg P $ha^{-1}$ with significant linear relationship with P application ($R^2 = 0.99$). There was also a positive linear relationship between P applied to preceding faba beans and wheat biomass P ($R^2 = 0.81$) and grain P uptake ($R^2 = 0.98$). The incorporated legume root, nodule and straw might have played a role in improving wheat yield through N addition via BNF and straw P addition. Therefore, we argue that the N and P benefits to rotational wheat are probably due to mineralization of crop residue derived organic N and P. The study demonstrates the prospects and importance of faba beans to be suitable alternative grain legumes for sustainable wheat based cropping systems in the humid tropical highlands of Ethiopia.

Key words: Faba bean–wheat rotation; $N_2$ fixation; N balance; N & P benefits; Wheat yield

## 4.1. Introduction

Nitrogen (N) and phosphorus (P) deficiency limits crop growth and grain yield in most tropical soils (Naab et al., 2009; Belane and Dakora, 2010). Traditional soil fertility restoring mechanisms like natural fallows are no longer options due to high population pressure (Vanlauwe et al., 2012). The use of chemical fertilizers could be a means to alleviate low nutrient levels and improve crop yields. However, chemical fertilizers are generally expensive for resource-poor farmers in SSA. A complementary strategy to increase soil fertility is the inclusion of $N_2$-fixing and P efficient grain legumes as bio-fertilizers in traditional cropping systems (Belane and Dakora, 2010), e.g. by growing them in rotation with cereals.

Several researchers have reported positive legume rotation effects on subsequent cereal yields (e.g. Carsky et al., 1997; Jemo et al., 2006). This beneficial effect has been mainly attributed to the availability of extra N through biological nitrogen fixation (BNF) (Dakora and Keya 1997; Habtegebrial and Sing, 2006; Habtemichial et al., 2007; Sanginga et al., 2002). This has been previously shown to be effective for soybean-maize (Jemo et al., 2006; Yusuf et al., 2009a), cowpea-maize (Adjei-Nsiah et al., 2008; Yusuf et al., 2009a), field pea-wheat (Habtegebrial and Singh, 2006), faba bean-wheat (Habtemichial et al., 2007) rotations.

Among legumes, faba bean (*Vicia faba* L.) is of great importance in legume-cereal rotations in cropping systems, where it is used as a break crop for cereals like wheat, barley and maize (Amanuel et al., 2000; Lopez-Bellido et al., 2006) and has the potential to enhance N and P nutrition of cereals when grown in rotation (Habtemichial et al., 2007; Nuruzzaman et al., 2005). Faba bean can improve the economic value of a subsequent cereal crop by enhancing the yield and/or increasing the protein content of the grain (Lopez-Bellido et al., 1998).

Increased levels of N and P in the soil after faba bean cropping and increased N and P uptake by cereal crops following faba bean can result from addition of N via BNF and mobilization and recycling of the sparingly available soil P (Turpin et al., 2002; Jensen et al., 2010; Peoples et al., 2009a).

Improvements in the cereal crop performance after legumes are not solely due to improvements in N availability. Legumes can provide a range of other potential rotational benefits that are not directly related to N such as enhanced P availability (Jemo et al., 2006; Nuruzzaman et al., 2005; Pypers et al., 2007), favorable microbial community in the rhizosphere (Marschner et al., 2004; Yusuf et al., 2009b) and breaking soil-borne disease cycles (Jensen et al., 2010). Horst et al. (2001) and Kamh et al. (1999) have also shown that growth and P uptake of subsequent maize were improved due to mobilization of soil P by P-efficient legume crops.

During the growth of faba bean, a high amount of $N_2$ is fixed often resulting in a positive N balance when crop residues are incorporated in the soil after grain harvest. Net N gains due to residue incorporation of about 84 kg N $ha^{-1}$ have been reported (Amanuel et al., 2000; Maidl et al., 1996). Several studies reported savings of up to 100-200 kg N $ha^{-1}$ in the amount of N-fertilizers applied to cereals following faba beans. For example, the residual N benefit to wheat from a previous sown faba bean was found to represent a saving of 30 kg fertilizer N $ha^{-1}$ compared to a wheat-wheat rotation (Jensen et al., 2010; McEwen et al., 1989). A rotation-study in Canada comparing a faba bean-barley-wheat and a barley-barley-wheat rotation showed that faba bean enhanced the average yield in the subsequent barley and wheat crops by 21 and 12%, respectively, which was equivalent to providing the cereals with around 120 kg N $ha^{-1}$ of N fertilizer (Wright, 1990). Kirkegaard et al. (2008) and Habtemichial et al. (2007) have also found wheat yield increases of 20 - 36% in the faba bean-wheat rotation compared to a barley-wheat rotation. For such a positive effect to occur, the amount of fixed N returned by the legumes to the soil should be greater than the amount of soil N in the harvested grain (Giller and Wilson, 1991).

Where the bulk of the above ground biomass of the legume component is removed from the system, the net N contribution to the soil may be negative and so yield increase in the subsequent cereal crop, if any, must be caused by other rotational effects. Such rotational effects have been observed for several other legumes. Sanginga et al. (2002) recorded yield increase in maize following soybean and Habtegebrial and Singh (2006) in wheat following field pea where the N balances in both cases were -8 kg ha$^{-1}$ and -9 kg ha$^{-1}$, respectively suggesting that other rotational effects other than N are operating. In addition to possible enhancement of N and P supply to cereals grown in rotation, improvement of soil physical properties, nematode suppression and high mycorrhizal infection shave been reported to contribute to a positive rotational effects of legumes (Bagayoko et al., 2000; Jemo et al., 2006). There is also a notion that grain legume-cereal rotation contributes less to soil fertility because of a lower plant biomass and higher nutrient removal by the legume grain at harvest (Jemo et al., 2006). However, grain legumes are more attractive to the resources poor farmers in SSA for food and cash income (Sanginga, 2003).

Faba bean-wheat rotation is a traditional cropping system in the highlands of Ethiopia. However, farmers usually use local varieties and remove faba bean biomass (crop residue) from the field during harvesting. Moreover, information is lacking whether high BNF and P efficient faba bean varieties in P limited soils of Ethiopia are beneficial to improve the grain yield of wheat subsequently grown in rotation. On top of that, abandonment of natural fallows and the limited access to mineral fertilizers make the inclusion of grain legumes for resources poor farmers essential. The exploitation of high BNF and P efficient legumes in cropping systems is emerging as an alternative and/or complementary strategy to the application of mineral fertilizers for sustainable agricultural intensification of low available nutrient cropping systems (The Montpellier Panel, 2013). Thus, the objective of this study was to examine to which extent high BNF and P efficient faba bean varieties lead to positive effects on wheat grown in rotation.

## 4.2. Materials and methods

### 4.2.1 Field sites

The study was conducted on three different farmers' fields in Dedo, located in southwestern Ethiopia at 7°28'48" N and 36°52'19"E at an elevation of 2,160 m above sea level in the 2009-2010 and 2010-2011 cropping seasons. The first two sites were previously cropped to wheat and the third site to teff. The mean annual temperature of the study area is 20 ± 0.2 °C and average yearly rainfall is ca. 1874 ± 77 mm (data from 1975-2011, obtained from the National Meteorology Agency of Ethiopia). Total rainfall in 2010 was 1470 mm and 1717 mm in 2011. Most of the rain (ca.73 %) falls in the months of April-September (Figure 4.1). The average minimum and maximum temperature for 2010 was 12.3 and 22 °C and 12.7 and 23.5 °C in 2011, respectively. Soils were classified as *Nitisols* in the FAO/WRB system. Top soils in the three fields were high in clay (53 - 55%), silt (43 - 45%), and total organic carbon (2.4 - 3.1%), but were acidic (pH-$H_20$ 5.5 - 5.8) and low in available P (Bray I method, Bray and Kurtz, 1945) 7 - 12 mg P $kg^{-1}$)

(Table 4.1). Participatory rural appraisal (PRA) research indicated that the cropping system is characterized by cereal (wheat, teff, barley) and legume (faba bean, field peas) production.

### 4.2.2 The first crop phase

In the first crop phase (2009/10 cropping season), three improved faba bean varieties (*Degaga*, *Moti* and *Obse*), one locally used faba bean variety, and wheat (*T. aestivum*) were grown as first crop in the legume-cereal rotation. The three improved faba bean varieties were selected because they had good adaptation potential, highest grain yield, highest BNF, positive soil N and high P utilization efficiency among six faba bean varieties tested in the 2008-2009 cropping season at the same study area. The local faba bean variety is characterized by low yields, susceptibility to foliar fungal diseases (e.g., chololate spot, leaf rust) and is sensitive to weed competition (Agegnehu and Fessehaie, 2006). Also, the wheat is a locally adapted variety introduced in the region long ago and produced widely by smallholder farmers. Its yield potential is estimated at ca 1.5 - 2.0 t ha$^{-1}$ (Personal communication with local farmers). Like the faba bean, wheat is also very sensitive to weed competition.

The experiment was laid out in a split-plot design with main plots measuring 60 m$^2$ (15 × 4 m) and the sub plots 12 m$^2$ (4 × 3 m). All treatment combinations were replicated in three farmers' fields selected in the cereal-legume cropping area of Dedo. These three fields thus constituted three randomized complete blocks. The main plots received the improved varieties (*Degaga*, *Moti* and *Obse*) and the sub plots P fertilizer in the form of *ORGA* at the rate of 0, 10, 20 and 30 kg P ha$^{-1}$. Both the main plot and sub plots were randomized. The local faba bean and wheat reference plots were not part of the split plot but included in each farm at the size of sub plot level as local control plots. No P fertilizer treatment was applied to the local faba bean and the wheat reference crops.

The term "*ORGA*" represents a trade name of organic fertilizer, which is locally manufactured by National Fertilizer Manufacturing Pvt. Ltd. Co. (NAFMAC) and contains 1% N + 23% $P_2O_5$ which provides substantial amounts of phosphorus. Additionally, "*ORGA*" contains 21% organic matter and 21% calcium oxide per 100 kg. Basically, "*ORGA*" fertilizer is made up of bones, stomach paunch, horns and hooves (NAFMAC, 2002) hence it could be included under the group of sparingly soluble fertilizers like ground phosphate rock and bone meal, with phosphorus present as tricalcium phosphate, $Ca_3(PO_4)_2$. The *ORGA* fertilizer was broadcast and incorporated thoroughly in the top 15 cm before sowing.

The wheat and the local faba bean were sown following farmers' agronomic practices. The faba bean seeds were sown by broadcasting at a rate of 200 kg grains $ha^{-1}$ (ca. 25 plants $m^{-2}$). The reference wheat seeds were sown by broadcasting at a rate of 120 kg $ha^{-1}$. Faba bean seeds were obtained from the breeding center for faba beans at Holeta Agricultural Research Center of the Ethiopian Institute of Agricultural Research. The faba bean seeds were not inoculated with rhizobia because they have been proven to nodulate effectively without inoculation in the previous years. The date of sowing of both the faba bean and wheat was July 23, 2010 and harvested in November 24 - 25, 2010. Only the pods were harvested from the faba bean plots and the wheat was mowed 5 - 10 cm above the ground following farmers' traditional practices. After the faba bean and wheat harvest the experimental area was fenced to protect dislodging of the crop residue by cattle and people.

#### *4.2.2.1 Plant sampling and analyses*

During late flowering, five faba bean plants were randomly selected and uprooted from the center of each subplot and plant parts separated into root and haulm components. Soil adhering to the roots was removed by washing with tap water. The nodules from each plant were removed, counted and the nodule dry weight (NDW kg $ha^{-1}$) was measured. The plots were additionally sampled for yield measurement at grain physiological maturity hence, plants from an area of 1 $m^2$ of each subplot were harvested, subsequently separated into grains and crop residues (roots, haulms and empty pods) and dried at 70 °C for 48 hours to determine root dry weight (RDW kg $ha^{-1}$), haulm dry weight (SDW t $ha^{-1}$), grain dry weight (GDW t $ha^{-1}$), empty pod dry weight (PDW t $ha^{-1}$) and total biomass yield (TBY t $ha^{-1}$ = NDW + RDW + SDW + PDW + GDW) on dry weight basis. A subsample of about 300 g was milled to pass through a 0.5 mm sieve for the determination of total N, $\delta^{15}N$ and P content of samples.

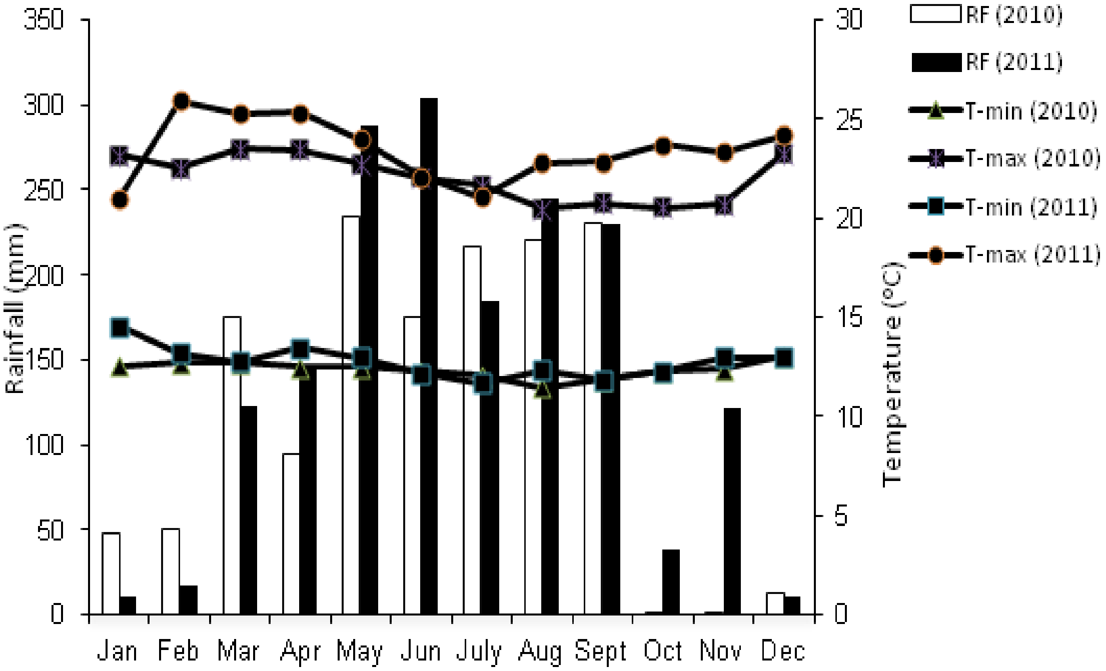

Figure 4.1. Total monthly rainfall (RF), average minimum (T-min) and maximum (T-max) temperatures of the Dedo in 2010 and 2011.

Total N and $\delta^{15}$N in plant samples was analyzed with an Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). P concentration (%) in plant samples was determined according Chapman and Pratt (1961) using slight modifications according to Ryan et al. (2001). Plant samples (0.5 – 1.0 g) were ashed in porcelain crucibles for five hours at 550 °C. The ash was dissolved in 5 mL 2N HCl and milli-Q water was added until a volume of 25 mL. The solution was filtered through Whatman No. 5 filters. P in filtrates was analyzed with an auto-analyser (Autoanalyzer 3, Bran+Luebbe, Norderstedt, Germany).

Table 4.1. Mean ± standard deviation of physicochemical soil characteristics of the study site on farmers' fields in Dedo

| | $pH_{H20}$ | $pH_{KCl}$ | TC | TN | TP | Available P | Clay | Silt | Sand | CEC | Ca | Mg | Na | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Farm | | | (%) | (%) | -------(mg P $kg^{-1}$)------- | | --------------- (%) ---------------- | | | -----------------------(cmol$_c$ $kg^{-1}$)--------------------- | | | | |
| 1 | 5.8±0.1 | 5.3±0.1 | 3.1±0.1 | 0.31±0.1 | 1130±99 | 6.7±1.8 | 53.7±1.5 | 44.0±1.7 | 2.3±0.6 | 38.8±0.3 | 15.5±0.3 | 2.8±0.1 | 0.07 | 1.5±0.1 |
| 2 | 5.6±0.1 | 5.2±0.1 | 3.0±0.1 | 0.31±0.1 | 1156±18 | 10.7±2.5 | 53.3±3.1 | 44.7±3.1 | 2.0±0.1 | 36.5±1.4 | 12.6±0.1 | 2.3±0.1 | 0.07 | 1.3±0.2 |
| 3 | 5.5±0.01 | 5.0±0.1 | 2.4±0.2 | 0.25±0.1 | 1284±81 | 12.3±4.7 | 55.3±1.5 | 42.6±1.5 | 2.0±0.1 | 36.7±2.4 | 13.4±0.4 | 2.7±0.3 | 0.07 | 1.0±0.1 |

TC = Total C, TN = total N, TP = total P

#### 4.2.2.2 N accumulation, BNF and N derived from soil

After determination of total N in the plant samples, nitrogen accumulation (N yield) of each plant part was calculated as: N yield (kg N $ha^{-1}$) = dry mater yield (kg $ha^{-1}$) × %N/100. The percentage of plant-N derived from atmospheric $N_2$ (%Ndfa), was calculated based on $^{15}N$ measurements in faba beans and reference plant by the $^{15}N$ natural abundance method (Peoples et al., 2009b) as:

$\% \text{ Ndfa} = 100 \times (\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{legume}}) / (\delta^{15}N_{\text{reference plant}} - \textit{B-value})$

Where, $\delta^{15}N_{\text{reference plant}}$ and $\delta^{15}N_{\text{legume}}$ are the whole plant $\delta^{15}N$ values of wheat and faba bean, respectively. The *B* value is the $\delta^{15}N$ value of faba beans grown in sterilized vermiculite for 47 days (flowering stage) with a nutrient solution free of N in a growth chamber (Vincent, 1970). The *B-value* was determined for each faba bean variety in a previous *B-value* study (Nebiyu et al., 2013). The amount of $N_2$ fixed by the legume (BNF) was calculated as BNF (kg N $ha^{-1}$) = total N yield × (%Ndfa)/100 and the N harvest index (NHI) as NHI (%) = total grain N (kg $ha^{-1}$)/total N yield (kg $ha^{-1}$) ×100. The N derived from soil (N from soil) was calculated as N from soil (kg N $ha^{-1}$) = total N yield (kg N $ha^{-1}$) – BNF (kg N $ha^{-1}$).

#### 4.2.2.3 N balance

A simple input/output model was used to calculate the N balance. The input was total BNF, seed N and N from fertilizer while the output was all N removed via harvested products. Though the amount of N added with ORGA fertilizer is very small compared to the amount of N fixed, it was taken as N input. Given the high seed N content and seed weight (Table 2.1), the seed N was also taken as N input. In the balance, two possible scenarios were examined. In the first scenario

(scenario-I), it was assumed that all the aboveground biomass is exported from the field by the farmers: N balance-I (kg N ha$^{-1}$) = (BNF, kg N ha$^{-1}$ + seed N, kg N ha$^{-1}$ + N from fertilizer, kg N ha$^{-1}$) – N removed (kg N ha$^{-1}$) via haulms, empty pods and grains. In the second scenario (scenario-II), all the above ground biomass except grains and empty pods is returned to the soil (N balance-II (kg N ha$^{-1}$) = (BNF, kg N ha$^{-1}$ + seed N, kg N ha$^{-1}$ + N from fertilizer, kg N ha$^{-1}$) – N removed (kg N ha$^{-1}$) via empty pods and grains.

#### *4.2.2.4 P accumulation in plant parts and P harvest index (PHI)*

P accumulation (kg ha$^{-1}$) in nodules, roots, haulms, empty pods and grains was determined as P concentration (%) in the dry biomass of each plant part × dry weight of the biomass (kg ha$^{-1}$). P harvest index in % (PHI, proportion of P exported via grains) was calculated as PHI (%) = grain P accumulation/total P accumulation × 100.

### 4.2.3 The second crop phase

In April 2011, when farmers in the region start land preparation for wheat, the crop residue of the three faba bean varieties (*Degaga*, *Moti* and *Obse*) that were left on the plots were cut into pieces, hand plowed and incorporated (this residue retention is denoted henceforth with the symbol ‘+’) into the respective plots by using a hoe. The crop residue of the local faba bean variety and that of the reference wheat was not retained (denoted with symbol ‘-’), i.e. it was taken away by the farmer during harvesting in November to simulate the common farmers’ traditional practices in the region. Wheat was grown on all plots that had faba bean and wheat in the first crop phase in order to test the beneficial role of faba varieties and *ORGA* fertilizer rates for the subsequent wheat. Wheat was sown by broadcasting at a rate of 120 kg $ha^{-1}$. No P and N fertilizers were applied to the succeeding wheat crop. At grain maturity, the wheat was harvested and grain (t $ha^{-1}$), straw (t $ha^{-1}$) and total biomass (t $ha^{-1}$) yield and N (kg $ha^{-1}$) and P (kg $ha^{-1}$) uptakes were determined as in the first crop phase.

### 4.2.4 Statistical analysis

In order to understand the main effect of each factor (variety and P fertilizer) and their interactions, if any, data from the first and second crop phases were subjected to analysis of variance using the general linear model (GLM) procedure of Statistical Analysis System (SAS) software version 9.2 (SAS, 2008) excluding the control plots (local faba bean and wheat) from ANOVA. The data were first checked for normality using the Proc univariate, option Normal procedure of SAS. Quantile-Quantile plot (Q-Q plot) on the residuals from ANOVA was taken into consideration for assessing the normality of the residuals. Due to lack of significant variety by P interaction ($P > 0.05$) for each of the response variables studied, interactions were excluded; and only the main effects of factors (variety and P) are presented in this thesis. When the F-test denotes significant effect of the main effect on a response variable, the least significant difference (LSD) was used to compare means at $P < 0.05$. For the first crop phase (the legume phase) the main effect of improved varieties was compared with the local faba bean and for the second crop phase (the wheat phase) comparison was also made with the wheat reference plot. In the wheat phase, the grain and biomass yield advantage of wheat over the local faba and wheat control plots was calculated based on the main effect values of the improved varieties. Although there was no significant interaction between the faba bean varieties and P applied during the first crop phase, correlation analysis was performed to establish interrelationships between P applied to faba beans during the first pase and the performance of the subsequent wheat.

## 4.3. Results

### 4.3.1. Performance of the preceding faba bean varieties

#### *4.3.1.1 Grain and biomass yield*

Haulm and total biomass yield of faba beans was significantly ($P < 0.05$) different between varieties (Table 4.2). Nodule, pod and grain biomass did not show differences due to varieties. *Moti* produced the highest haulm (4.5 ± 0.2 t $ha^{-1}$) and total biomass yield (10.3 ± 0.3 t $ha^{-1}$) of the three improved varieties. Faba bean grain yield ranged between 3.9 ±0.3 and 4.8 ± 0.8 t $ha^{-1}$ with no significant differences among the varieties. P application, on the other hand, did not significantly ($P > 0.05$) affect the grain and biomass yield of faba beans in the first crop phase. The variety by P level interaction was not statistically significant ($P > 0.05$). The average grain and total biomass yield of the reference wheat was 3.1 ± 0.5 and 4.6 ± 0.6 t ha-$^{1}$, respectively.

#### *4.3.1.2 N accumulation, BNF and N derived from soil*

The ANOVA showed that total N yield, %Ndfa, BNF and N derived from soil were significantly ($P < 0.05$) affected by faba bean variety (Table 4.3). P application did not result in any significant change; and there was no significant interaction effect between variety and P levels for N yield, %Ndfa, BNF and N from soil. Estimates of %Ndfa by the $^{15}N$ natural abundance method ranged from 71.8 ± 1.5% for variety *Degaga* to 94 ± 2.1% for *Moti* with significant difference among the varieties. The total amount of N yield by the varieties ranged from 345.3 ± 15.7 kg N ha$^{-1}$ for *Obse* to 409.0 ± 14.0 kg N ha$^{-1}$ for *Moti* (Table 4.3), and BNF in the whole plant ranged from 258.1 ± 16.8 (*Degaga*) to 387.0 ± 14.8 kg ha$^{-1}$ (*Obse*), with significant differences ($P < 0.05$) among varieties (Table 4.3). The amount of N derived from soil was also significantly ($P < 0.05$) varied between 22.0 ± 8.7 (*Moti*) and 97.8 ±5.8 kg ha$^{-1}$ (*Degaga*). The total soil N uptake of the reference wheat was 98.7 ± 15.7 kg ha$^{-1}$.

Table 4.2. Dry biomass yield (mean ± SE) of different plant parts (nodules, roots, haulms, empty pods and grain) of faba bean varieties and wheat and P application levels. The values are means ± SE. Means followed by different letters in a column are significantly different from each other at $P < 0.05$ according to the LSD test.

| | Biomass yield | | | | | |
|---|---|---|---|---|---|---|
| Variety | Nodule (g plant) | Root (kg ha$^{-1}$) | Haulm (t ha$^{-1}$) | Empty Pod (t ha$^{-1}$) | Grain (t ha$^{-1}$) | Total biomass (t ha$^{-1}$) |
| *Degaga* | 0.24 ±0.02a | 342.7 ±19.8b | 3.7±0.3b | 0.90 ±0.1 | 4.3 ±0.3 | 9.2±0.4b |
| *Moti* | 0.24 ±0.02a | 387.2 ±16.5a | 4.5±0.2a | 0.97 ±0.04 | 4.4 ±0.2 | 10.3±0.3a |
| *Obse* | 0.22 ±0.01a | 388.2 ±20.4a | 3.9±0.1b | 0.99 ±0.1 | 3.9 ±0.3 | 9.2±0.4b |
| F-test | * | * | * | ns | ns | * |
| $LSD_{0.05}$ | 0.05 | 47.8 | 0.6 | 0.2 | 0.9 | 1.2 |
| Reference crops (control) | | | | | | |
| Local faba | 0.15 ±0.01 | 410.8 ±60.7 | 4.0±0.8 | 1.10 ±0.1 | 4.8 ±0.8 | 10.4±0.4 |
| Wheat | - | - | 1.5±0.1 | - | 3.1±0.5 | 4.6±0.6 |
| P-levels (kg ha$^{-1}$) | | | | | | |
| 0 | 0.23±0.02 | 401.9±23.2 | 4.1±0.3 | 0.98±0.1ab | 4.5±0.4 | 10.1±0.4 |
| 10 | 0.23±0.02 | 363.5±14.8 | 3.9±0.2 | 0.98±0.1ab | 3.8±0.4 | 9.2±0.3 |
| 20 | 0.20±0.01 | 364.8±21.9 | 3.8±0.3 | 0.83±0.1b | 3.9±0.2 | 9.0±0.4 |
| 30 | 0.25±0.02 | 363.5±26.7 | 4.2±0.3 | 1.1±0.1a | 4.5±0.3 | 10.2±0.6 |
| F-test | Ns | ns | ns | * | ns | ns |
| $LSD_{0.05}$ | 11.6 | 41.4 | 0.5 | 0.2 | 0.8 | 1.1 |

*= significant at $P < 0.05$; ns = not significant

The N yield per plant part and N harvest index (NHI) was also calculated to assess the partitioning of fixed $N_2$ into the different plant parts. ANOVA showed that, except N yield of empty pods and grains and NHI, N yield of nodules, roots and haulms was significantly different for varieties (Table 4.3). Here also, P application and the variety by P interaction did not result in any statistically significant change in N accumulation by faba bean plant parts except for root and empty pod N yield. Faba bean haulms had highest N yield (kg N $ha^{-1}$) in the range between 137.1 ± 10.3 (*Degaga*) and 177.6 ± 8.7 (*Moti*) that corresponds to 39 and 44 % of the total N fixed. Grains had also the highest N yield ranging between 177.3 ± 15.2 (*Obse*) and 194.3 ±15.4 (Degaga) which corresponds respectively to 51 and 54% of the total N fixed with no significant difference among the varieties. These results indicated different partitioning of fixed N between plant parts and that the greater fraction of fixed N was translocated to haulms and grains and hence the NHI of faba beans ranged from 49.4% to 54.1% with, however, no significant difference between varieties. The NHI of the reference wheat was 75.3 ± 2.3% which was much higher than the NHI of faba bean varieties.

#### *4.3.1.3. The N balance*

The N budget (kg N $ha^{-1}$) was calculated for two crop residue management scenarios (scenario-I and scenario-II) for the faba bean varieties as shown in Tables 4.4. Exporting all aboveground biomass (scenario-I) gave a negative net N balance (kg $ha^{-1}$) in the range of -78.4 ± 6.1 (*Degaga*) to -2.8 ± 8.7 (*Moti*) with significant differences between varieties. Scenario-I assumes that N is recycled only via underground plant parts (nodules and roots) and the amount of N recycled ranged from 10.1 ± 0.7 (local faba) to 12.6 ± 0.6 (*Moti*), which is far below that removed by above ground plant biomass in the range 334.6 ± 15.9 (*Obse*) to 396.4 ± 14.2 (*Moti*). Calculations of the net soil N balance assuming that only the empty pods and grains were exported (scenario-II) showed that all balances were significantly ($P < 0.01$) improved and all the varieties were positively contributing N to the system. Further in this scenario, the calculated N balance ranged from 58.7 ± 13.4 (*Degaga*) to 174.8 ± 13.7 (*Moti*) kg N ha$^{-1}$ with significant differences between varieties. The N removed in scenario-II were not significantly ($P > 0.05$) different between varieties. Here also, P application and the variety by P interaction did not affect the N balance of either of the scenarios (Tables 4.4).

Table 4.3. Total N yield, N sources, N accumulation (kg N $ha^{-1}$) in plant parts (nodules, roots, haulms, empty pods and grain) and the N harvest index (NHI, %) of faba bean varieties and wheat and P application levels. The values are means ± SE. Means followed by different letters in a column are significantly different from each other at $P < 0.05$ according to the LSD test. The N yield and N accumulation measurements are based on the dry yield.

|  |  |  | N sources |  | N accumulation in plant parts |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Total N yield |  | -----------(kg $ha^{-1}$)------------ |  | ------------------------------(kg $ha^{-1}$)---------------------------- |  |  |  |  | NHI |
| Variety | (kg $ha^{-1}$) | %Ndfa | N From BNF | N from Soil | Nodules | Roots | Haulms | Empty pod | Grain | (%) |
| *Degaga* | 356.0±17.9b | 71.8±1.5c | 258.1±16.8c | 97.8±5.8a | 4.5±0.3a | 6.8±0.3b | 137.1±10.3b | 13.2±0.9 | 194.3±15.4 | 54.1±2.7 |
| *Moti* | 409.0±14.0a | 94.5±2.1a | 387.0±14.8a | 22.0±8.7b | 4.8±0.5a | 7.8±0.3ab | 177.6±8.7a | 15.3±1.4 | 203.5±12.5 | 49.4±2.1 |
| *Obse* | 345.3±15.7b | 81.7±1.6b | 274.5±14.2bc | 70.7±12.5a | 3.9±0.2a | 7.2±0.3ab | 142.9±6.1b | 13.9± 1.2 | 177.3±15.2 | 50.5±2.5 |
| F-test | * | * | * | * | * | * | * | ns | Ns | ns |
| $LSD_{0.05}$ | 50.1 | 7.5 | 47 | 34.0 | 1.3 | 1.1 | 31.2 | 4.0 | 47 | 7.6 |
| Reference crops (control) |  |  |  |  |  |  |  |  |  |  |
| Local faba | 393.8±21.2 | 75.7±12.1 | 311.6±27.6 | 82.3±34.9 | 2.0±0.9 | 8.1±1.2 | 157.3±35.0 | 15.5±1.3 | 211.3±36.4 | 53.5±8.1 |
| Wheat | 98.7±15.7 | - | - | 98.7±15.7 | - | - | 22.8±2.5 | - | 75.8±13.7 | 75.3±2.3 |
| P-levels (kg $ha^{-1}$) |  |  |  |  |  |  |  |  |  |  |
| 0 | 387.4±18.7 | 82.9±4.1 | 323.8±23.8 | 63.6±13.8 | 4.5±0.5 | 7.8±0.4a | 156.4±13.3 | 13.4±1.4ab | 205.3±15.9 | 52.5±3.0 |
| 10 | 345.8±14.5 | 83.2±4.5 | 287.8±19.6 | 57.9±15.8 | 4.7±0.4 | 7.3±0.4ab | 145.1±6.3 | 13.9±0.8ab | 174.7±18.4 | 49.6±3.4 |
| 20 | 351.8±16.9 | 82.6±3.9 | 294.3±25.9 | 57.5±12.5 | 3.7±0.2 | 7.3±0.3ab | 149.8±11.8 | 11.9±1.1b | 179.1±12.5 | 50.9±2.6 |
| 30 | 386.6±21.4 | 81.9±2.8 | 320.2±27.9 | 66.4±17.5 | 4.7±0.5 | 6.6±0.5b | 158.8±11.9 | 15.4±1.3a | 214.2±15.6 | 55.9±2.2 |
| F-test | Ns | ns | Ns | Ns | Ns | * | ns | * | Ns | ns |
| $LSD_{0.05}$ | 46.2 | 4.2 | 40.3 | 18.6 | 1.1 | 0.8 | 24.5 | 3.1 | 40.0 | 8.7 |

Table 4.4. The N budget (kg N ha$^{-1}$) of faba beans varieties and P application levels following two different scenarios. Scenario-I considers that N is recycled only via nodules and roots and scenario-II via nodules, roots and haulms. The values are mean ± SE. Means followed by different letters in a column are significantly different from each other at $P < 0.05$ according to the LSD test. In both scenarios BNF, N-via seed and N-via fertilizer were the N inputs.

| | N balance (kg ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | ----------------Scenario-I------------------ | | | --------------------Scenario-II----------------------------- | | |
| Variety | N removed-I (via haulms, empty pods and grains) | N recycled (via nodules and roots) | N balance-I (BNF + seed N + fertilizer N) – (N removed-I) | N removed-II (via empty pods and grains) | N recycled (via nodules, roots and haulms) | N balance-II (BNF +seed N + fertilizer N) – (N removed-I) |
| *Degaga* | 344.6 ±17.7b | 11.4 ±0.5ab | -78.4 ±6.1c | 207.5 ±15.9 | 148.5 ±10.7b | 58.7 ±13.4b |
| *Moti* | 396.4 ±14.2a | 12.6 ±0.6a | -2.8 ±8.7a | 218.8 ±13.1 | 190.2 ±8.6a | 174.8 ±13.7a |
| *Obse* | 334.6 ±15.9b | 11.1 ±0.4b | -48.3 ±12.6b | 191.3 ±16.1 | 154.0 ±6.3b | 94.6 ±13.0b |
| F-test | * | * | * | Ns | * | * |
| $LSD_{0.05}$ | 52.3 | 1.4 | 27.1 | 49.0 | 32 | 42.3 |
| Reference crop (control) | | | | | | |
| Local faba | 383.8 ±21.8 | 10.1 ±0.7 | -67.0 ± 35.2 | 226.5 ±37.5 | 167.3 ±35.1 | 90.2 ±16.4 |
| P-levels (kg ha$^{-1}$) | | | | | | |
| 0 | 376.3 ±16.7ab | 11.7 ±0.5 | -42.8 ±14.0 | 219.7 ±16.8 | 168.4 ±13.5 | 113.6 ±18.4 |
| 10 | 333.8 ±18.4b | 12.0 ±0.7 | -37.8 ±16.3 | 188.6 ±18.4 | 157.2 ±6.8 | 107.4 ±21.0 |
| 20 | 340.8 ±13.2ab | 10.9 ±0.3 | -37.3 ±13.2 | 191.0 ±13.2 | 160.7 ±11.8 | 112.5 ±25.2 |
| 30 | 385.2 ±16.7a | 11.6±0.8 | -54.8 ±17.6 | 226.4 ±16.7 | 170.4 ±12.4 | 104.0 ±24.5 |
| F-test | * | Ns | ns | Ns | Ns | ns |
| $LSD_{0.05}$ | 43.3 | 1.2 | 31.3 | 41.5 | 27.8 | 48.9 |

#### *4.3.1.4 Total P accumulation and P exported by faba beans*

**P accumulation**

P accumulation per plant part (nodule, root, haulm, empty pod and grain) and total plant P yield were determined for the faba bean varieties and P application levels to assess the partitioning of P into the different plant parts (Table 4.5). ANOVA showed that varieties were significantly ($P < 0.05$) different for P accumulation for nodules, haulms and PHI. P application levels resulted in significant ($P < 0.05$) changes of P accumulation only in empty pods and total plant P yield. The variety by P application interaction did not result in any significant changes in P accumulation by plant parts or total plant P yield ($P > 0.05$). The local faba bean accumulated significantly lower P levels (0.19 ± 0.01 kg P $ha^{-1}$) in the nodules compared to the improved varieties. *Moti* contained significantly higher P levels in the haulms (14.4 ± 0.7 kg P $ha^{-1}$) compared to others. The total P yield ranged from 26.5 ± 1.4 to 30.8 ± 1.8 kg P $ha^{-1}$ with no significant differences between varieties. At 30 P applications, faba bean varieties accumulated significantly higher P in empty pods (0.97 ± 0.1 kg P $ha^{-1}$) and total plant (30.3 ± 1.8 kg P $ha^{-1}$).

**P exported by faba bean**

The P harvest index (PHI, proportion of P exported via grains and empty pods) was determined for the faba bean varieties and P application levels to assess the extent to which P is removed from the system. ANOVA showed that, only the variety factor affected the PHI and ranged 42.8 ± 2.2% (*Moti*) to 53.7 ± 6.8% (local faba) with a significant ($P <0.05$) difference between varieties (Table 4.5). The P application main effect and variety by P application interaction did not result in any significant changes in the PHI of faba beans.

Table 4.5. P accumulation (kg P ha$^{-1}$) in plant parts (nodules, roots, haulms, empty pods and grain) and P harvest index (PHI, %) of faba bean varieties and P application levels. The values are means ± SE, means followed by different letters in a column are significantly different from each other at P < 0.05 according to the LSD test. The P accumulation measurements are based on the dry yield.

| | P accumulation in plant parts | | | | | | |
|---|---|---|---|---|---|---|---|
| | ------------------------------------------(kg P ha$^{-1}$)------------------------------------------ | | | | | | PHI |
| Variety | Nodules | Roots | Haulms | Empty pod | Grain | Total | (%) |
| *Degaga* | 0.23 ±0.02ab | 0.65 ±0.03 | 11.3 ±0.8b | 0.60 ±0.04 | 13.7 ±1.1 | 26.5 ±1.4 | 51.3 ±3.0ab |
| *Moti* | 0.26 ±0.02a | 0.70 ±0.03 | 14.4 ±0.7a | 0.79 ±0.1a | 12.4 ±1.0 | 28.6 ±1.4 | 42.8 ±2.2b |
| *Obse* | 0.23 ±0.01ab | 0.69 ±0.03 | 11.5 ±0.3b | 0.77 ±0.10 | 14.0 ±1.2 | 27.2 ±1.4 | 50.5 ±2.1ab |
| F-test | * | Ns | * | ns | Ns | ns | * |
| LSD$_{0.05}$ | 0.06 | 0.1 | 2.8 | 0.3 | 4.2 | 5.0 | 10.5 |
| Reference crop (control) | | | | | | | |
| Local | 0.19 ±0.01 | 0.79 ±0.14 | 12.5 ±2.1 | 0.72 ±0.10 | 16.6 ±2.6 | 30.8 ±1.8 | 53.7 ±6.8 |
| P-levels (kg ha$^{-1}$) | | | | | | | |
| 0 | 0.25 ±0.02 | 0.74 ±0.04 | 12.7 ±0.7 | 0.61±0.04b | 14.5 ±1.3 | 28.8 ±1.4ab | 49.3 ±3.1 |
| 10 | 0.24 ±0.03 | 0.67 ±0.03 | 11.8 ±0.4 | 0.66 ±0.07b | 11.9 ±1.2 | 25.3 ±1.0b | 46.5 ±3.1 |
| 20 | 0.20 ±0.01 | 0.68 ±0.03 | 11.7 ±1.2 | 0.68 ±0.08b | 12.6 ±0.6 | 25.8 ±1.4b | 49.4 ±2.9 |
| 30 | 0.25 ±0.02 | 0.65 ±0.05 | 13.3 ±0.8 | 0.97 ±0.12a | 15.5 ±1.5 | 30.3 ±1.8a | 49.3 ±2.9 |
| F-test | ns | Ns | ns | * | Ns | * | Ns |
| LSD$_{0.05}$ | 0.05 | 0.12 | 2.4 | 0.22 | 3.6 | 4.3 | 8.2 |

*= significant at P <0.05; ns = not significant

## 4.3.2. Performance of succeeding wheat grown after faba beans in rotation

### *4.3.2.1. Biomass and grain yield of wheat*

Wheat was grown after the harvest of faba beans and the reference wheat in order to test whether P efficient and high $N_2$ fixing faba bean varieties are beneficial to subsequently grown wheat. No N or P was applied to the succeeding wheat. The biomass yield of wheat was only significantly ($P < 0.05$) affected by the main effect of pre-crop variety (faba beans in the first phase) but grain yield of wheat was significantly ($P < 0.05$) affected both by the main effect of pre-crop variety and P application levels. The pre-crop variety by P application interaction had no significant effect ($P > 0.05$) on total biomass and grain yield of wheat. The biomass and grain yield of wheat grown after the faba beans were improved significantly ($P < 0.05$) compared to the yield of wheat after wheat (Tables 4.6, 4.7). Total biomass yield of wheat in the rotation ranged significantly between 4.7 ± 0.9 (wheat-wheat rotation) to 9.9 ± 1.3 (*Moti*-wheat rotation) and wheat grain yield varied significantly between 1.5 ± 0.3 t $ha^{-1}$ (wheat-wheat rotation) to 2.8 ± 0.3 t $ha^{-1}$ (*Obse*-wheat rotation). These corresponded to a 112 and 82% biomass and grain yield increase relative to the yield of wheat after (Table 4.6).

Table 4.6. Effect of previous crops (faba bean varieties and wheat) on total biomass (TBY) and grain (GY) yield of rotational wheat and the percent yield advantage compared to rotation with local faba and wheat with above ground biomass removal in the humid tropical highland of Ethiopia. The total biomass and grain yield measurements are based on the dry yield. The values are means ± SE, (+) indicates that residue is incorporated and (-) shows not incorporated.

| | ------- TBY (t $ha^{-1}$)---------- | | | -------------- GY (t $ha^{-1}$)------------ | | |
|---|---|---|---|---|---|---|
| Previous crop | t $ha^{-1}$ | % increase over local faba(-) | % increase over wheat (-) | t $ha^{-1}$ | % increase over local faba(-) | % increase over wheat reference(-) |
| *Degaga*(+) | 5.9 ±0.7 | -3 | 27 | 1.8 ±0.2 | -9 | 18 |
| *Moti*(+) | 9.9 ±1.3 | 61 | 112 | 2.5 ±0.2 | 27 | 65 |
| *Obse*(+) | 7.3 ±0.9 | 18 | 55 | 2.8 ±0.3 | 40 | 82 |
| $LSD_{0.05}$ | 2.7 | | | 0.6 | | |
| Reference crops (control) | | | | | | |
| Local faba(-) | 6.2 ±2.1 | - | 31 | 1.9 ±0.2 | - | 29 |
| Wheat(-) | 4.7 ±0.9 | -24 | - | 1.5 ±0.3 | -21 | - |

P application to the preceding faba bean varieties also significantly improved the total biomass yield of wheat that ranged between 6.1 ± 0.9 (at 0 kg P) to 8.9 ±1.8 t $ha^{-1}$ (at 30 kg P) and the grain yield of the succeeding wheat between 1.9 ± 0.3 (at 0 kg P) to 2.8 ± 0.3 t $ha^{-1}$ (at 30 kg P) (Fig 4.2) with a linear relationship between P application to the previous faba beans and wheat biomass ($R^2$ = 0.97) and grain yield ($R^2$ = 0.97). The variety by P interaction was however not significant ($P > 0.05$).

Table 4.7. Pairwise comparison between previous faba bean varieties and reference scenario (local faba bean and wheat as preceding crop with above ground biomass removal) on total biomass (TBY) and grain (GY) yield production of rotational wheat in the humid tropical highland of Ethiopia. (+) indicates that crop residues were retained and (-) shows they were removed.

| | | ------------------TBY (t $ha^{-1}$)------------------- | | | ----------GY (t $ha^{-1}$)------------ | | |
|---|---|---|---|---|---|---|---|
| | | | 95% confidence limits | | | 95% confidence limits | |
| Pre-crop | Reference | [a]Mean difference | Lower | Upper | Mean Difference | Lower | upper |
| Degaga(+) | Local faba(-) | -0.19ns | -3.02 | 2.64 | -0.18ns | -0.80 | 0.43 |
| | Wheat(-) | 1.28ns | -1.55 | 4.41 | 0.26ns | -0.35 | 0.88 |
| Moti(+) | Local faba(-) | 3.75* | 0.92 | 6.58 | 0.54ns | -0.08 | 1.15 |
| | Wheat(-) | 5.22* | 2.39 | 8.05 | 0.99* | 0.37 | 1.61 |
| Obse(+) | Local faba(-) | 1.12ns | -1.71 | 3.95 | 0.80* | 0.18 | 1.42 |
| | Wheat(-) | 2.59ns | -0.24 | 5.42 | 1.25* | 0.63 | 1.87 |
| Local faba(-) | Wheat(-) | 1.47ns | -2.11 | 5.05 | 0.45* | -0.33 | 1.23 |

[a] comparisons significant at $P < 0.05$ are indicate by * and those not significant by ns.

#### *4.3.2.2. N and P uptake of wheat*

**N uptake**

Total plant and grain N uptake of wheat after faba beans was significantly ($P < 0.05$) different depending on the faba bean variety used (Table 4.8). The highest total plant N uptake (kg N $ha^{-1}$) was obtained from all faba bean plots in the range 48.6 ± 7.7 (*Degaga*) to 77.3 ± 6.7 (*Moti*) compared to the wheat reference plot (42.1 ± 7.9). Grain N uptake of wheat was also significantly different ($P < 0.05$) depending on the preceding crop.

The highest grain N uptake was obtained from pre-crop plots of *Obse* (57.8 ± 6.8 kg N $ha^{-1}$) and *Moti* (52.4 ± 5.4 kg N $ha^{-1}$) compared to the wheat-wheat plot (30.1 ± 1.1 kg N $ha^{-1}$) with significant differences among the precursor varieties. Further, P application levels to faba bean pre-crop varieties resulted in significant ($P < 0.05$) grain N uptake by the succeeding wheat. The highest grain N uptake (58.7 ± and 52.8 kg N $ha^{-1}$) was obtained at P application of 30 and 20 kg P $ha^{-1}$and the lowest grain N uptake (38.6 ± kg N $ha^{-1}$) at 0 kg P $ha^{-1}$ with significant linear relationship with P application ($R^2 = 0.99$) (Fig 4.3). The total plant N uptake of the succeeding wheat crop was, however, not significantly ($P > 0.05$) affected by P application levels and by variety by P interaction.

**P uptake**

ANOVA showed that total plant P uptake of wheat was significantly ($P < 0.05$) affected by the faba bean varieties. Compared to wheat grown after wheat, wheat after faba bean resulted in significantly higher total plant P uptake (kg P $ha^{-1}$) ranging from 6.7 ± 1.5 (*Degaga*) to 11.7 ±1.1 (*Moti*) compared to the wheat reference plot (5.4 ± 0.3) (Table 4.8). Wheat grain P uptake varied between 3.7 ± 0.7 to 7.2 ± 1.3 kg P ha$^{-1}$ with no significant differences among the previous crop or variety. P application to the previous crop (faba bean) and the variety by P level interaction did not bring a statistically significant effect on either total plant or grain P uptake of the succeeding wheat. However, there was a linear relationship between P applied and wheat biomass P ($R^2$ = 0.81) and grain P uptake ($R^2$ = 0.98) (Fig 4.4).

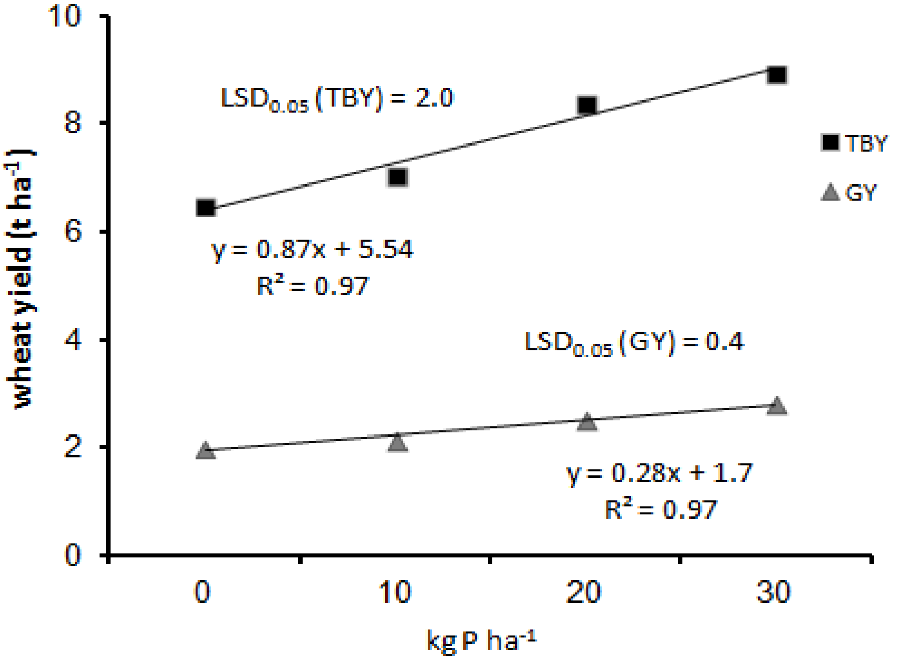

Figure 4.2. Effect of P applied to faba beans in the first crop phase on dry grain (GY) and total biomass (TBY) yield of rotational wheat on farmers field at Dedo, Southwest Ethiopia. The P levels significantly ($P < 0.05$) affected grain yield but did not significantly ($P > 0.05$) affect total plant biomass of wheat

Table 4.8. N and P yield of rotational wheat and the percent increase over the wheat–wheat rotation as affected by pre-crop faba bean varieties and wheat. The N and P yield measurements are based on the dry yield. The values are means ± SE, (+) indicates that crop residues were retained and (-) shows they were removed.

| | N yield (kg N ha$^{-1}$) | | | | P yield (kg P ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| Pre-crop | Grain | % increase over wheat(-) | Total plant | % increase over wheat(-) | Grain | % increase over wheat(-) | Total plant | % increase over wheat(-) |
| *Degaga*(+) | 38.3 ±5.1 | 27 | 48.6 ±7.7 | 15 | 4.0 ±1.3 | 8 | 6.7 ±0.9 | 24 |
| *Moti*(+) | 52.4 ±5.4 | 74 | 77.3 ±6.7 | 84 | 5.5 ±1.6 | 49 | 11.7 ±1.1 | 117 |
| *Obse*(+) | 57.8 ±6.8 | 92 | 55.9 ±12.7 | 33 | 7.2 ±1.3 | 95 | 8.2 ±2.3 | 52 |
| F-test | * | | * | | ns | | * | |
| $LSD_{0.05}$ | 14.2 | | 32.2 | | 3.7 | | 5.8 | |
| Reference crop (control) | | | | | | | | |
| Local faba(-) | 39.0 ±0.4 | 29 | 48.6 ±15.1 | 15 | 4.5 ±0.8 | 22 | 6.4 ±1.5 | 19 |
| Wheat(-) | 30.1 ±1.1 | - | 42.1 ±7.9 | - | 3.7 ±3.7 | - | 5.4 ±0.3 | - |

*= significant at P <0.05; ns = not significant

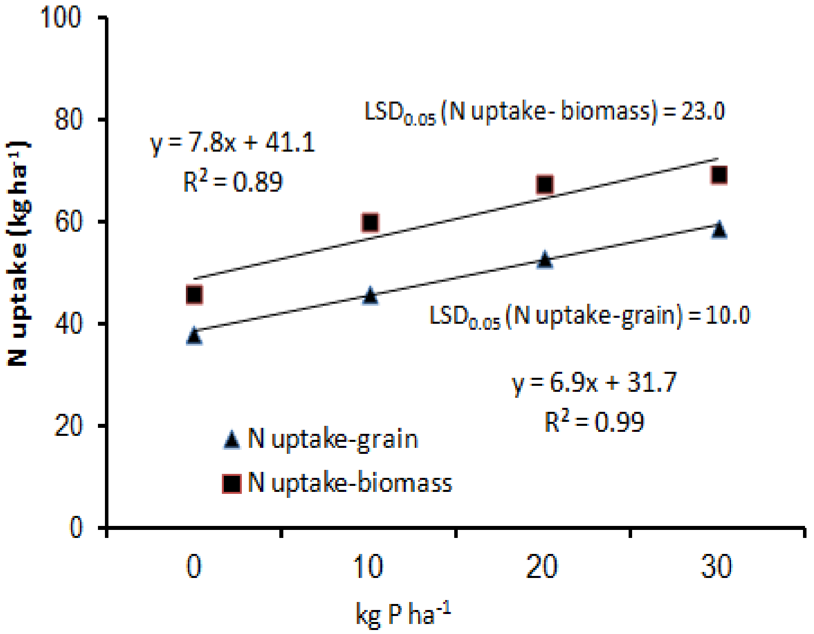

Figure 4.3. Effect of P applied to faba beans on grain N uptake (N uptake-grain) and total biomass N uptake (N uptake-biomass) of a rotational wheat. The P levels significantly ($P < 0.05$) affected grain N uptake but did not significantly ($P > 0.05$) affect total plant N uptake of wheat.

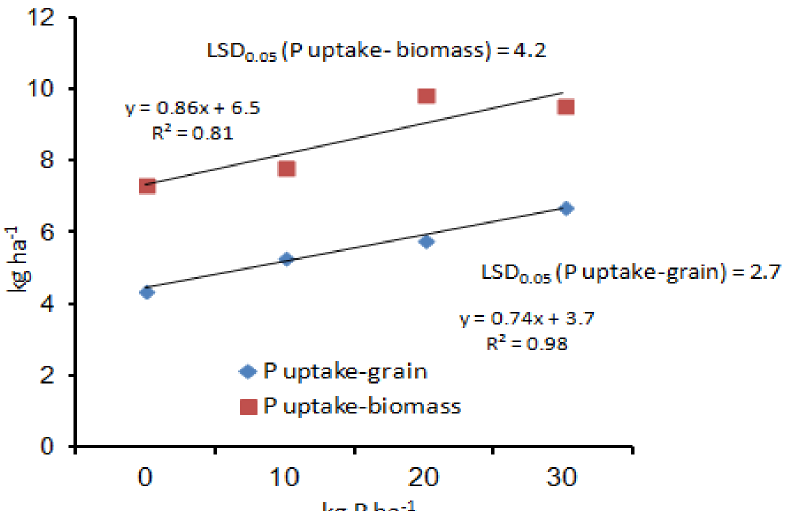

Figure 4.4. Effect of P applied to faba beans on grain P uptake (P uptake-grain) and total biomass P uptake (P uptake-biomass) of rotational wheat. The P levels did not significantly ($P > 0.05$) affect both grain P uptake and total plant P uptake of wheat. However, there was a positice correlation between P applied and P uptake-grain ($R^2$=0.98) and total biomass P uptake ($R^2$= 0.81).

## 4.4. Discussion

Rotational cropping involving legumes and cereals is a more sustainable system for increasing food production in Agro-ecosystems (Yusuf et al., 2009a). The faba bean-wheat rotation is a traditional cropping system in the highlands of Ethiopia. However, farmers usually use local varieties and traditionally remove all above ground faba bean biomass from the field during harvesting.

The present work demonstrated that the performance of faba bean varieties did not differ significantly for grain yield during the first crop phase. However, the varieties showed significant differences for haulm and total plant biomass yield. Variety *Moti* produced the highest haulm (4.5 t ha$^{-1}$) and total plant biomass yield (10.3 t ha$^{-1}$) compared to the other varieties. The superior haulm and total biomass production by *Moti* could be related to its high BNF potential (387 kg N ha$^{-1}$) and high P use efficiency (authors' unpublished data). P addition at different levels did not bring any significant difference in grain or total biomass yield of faba bean varieties, which suggested that the faba beans in the present study were not P limited.

The faba bean varieties showed variations in their proportion of plant N derived from fixation and soil. The total amount of N accumulated by the faba bean varieties ranged from 345 kg N ha$^{-1}$ to 409 kg N ha$^{-1}$ and the amount of total N fixed from 258 kg N ha$^{-1}$ to 387 kg N ha$^{-1}$ which are comparable with the reports of Köpke and Nemecek (2010) and Unkovich et al. (2008). The amount of N derived from soil by the varieties was also significantly different between varieties in the range 22.0 kg ha$^{-1}$ (*Moti*) and 82.3 kg ha$^{-1}$ (local faba bean) indicating that the faba bean varieties differ in their reliance on symbiotic N fixation. The significant variation in the ability to access the soil N pool would have a potential effect on soil N availability (N sparing effect) for uptake by a subsequent crop provided that there is no leaching of nitrate downwards out of the reach of the next crop. The NHI is also an indication of the soil N balance (Yusuf et al., 2009a) and the NHI of the present faba bean varieties varied from 49% to 54% with no significant difference between varieties but showed that considerable proportion of fixed N is translocated to grains. The results are comparable with the NHI of 49 – 55% reported for soybean (Yusuf et al., 2009a) and 41 - 59% for cowpeas (Adjei-Nsiah et al., 2008).

It is not in all cases that the legume cultivation results in a positive soil N balance. $N_2$ fixing legumes may not succeed to make a net positive contribution to soil N either due to a high NHI of the varieties or when %Ndfa is much less than N harvest index (Yusuf et al., 2009a). Simple N budgets of the faba bean varieties demonstrated large differences regarding potential N returns to soil. As shown in Table 4.4, the faba bean varieties could potentially deplete the soil with 2.8 to 78 kg N $ha^{-1}$, if all the above ground biomass is removed (scenario I). Thus, removal of faba bean grains and/or straw as practiced in Southwest Ethiopia is critical in determining the net contribution to soil N balance.

In low-input systems of SSA, crop residues are frequently removed from the field to be used as animal feed or as a source of energy (Adu-Gyamfi et al., 2007; Bakht et al., 2009), which is also taking place currently by smallholder farmers in the tropical highlands of Ethiopia. The main reason that farmers remove the crop residue from the field and transport to the house is that threshing is done at the homestead. The crop residue is partly fed to the livestock which may be beneficial to the entire system as some of the N may actually comeback as manure. However, part of the residue is still used as source of household energy. Further residue management options may be required for a more realistic understanding of the residue management strategies.

Aiming at changing this traditional practice, farmers need to be convinced via extension and demonstration activities to change their residue management practice from scenario I to scenario II so that soil N would be enriched positively and that crop residues would have more financial benefits when used as sources of plant nutrients (Bakht et al., 2009). On-farm demonstrations, popularization and scaling up of these practices using participatory approach may help farmers make an informed decision about the best crop residue management practices to apply.

The faba bean varieties in our study gave a large soil N surplus between 58 and 175 kg N ha$^{-1}$, depending on variety, which is equivalent to 126 – 380 kg ha$^{-1}$ of urea (46% N). The price of 1 kg urea fertilizer is on average ca. $1.6 in SSA (Chianu et al., 2011) and thus gives an economic advantage of $202 to 608 ha$^{-1}$ to the resource poor farmers. Even if the use efficiency of this extra N is less than that of fertilizer N this still represents a big advantage for subsistence cereal-based production systems in SSA, where fertilizers are too expensive or unavailable. BNF by faba beans can thus contribute to increased yields of subsequent cereals and therefore improve soil fertility if residues are incorporated to the soil.

Wheat was grown after the harvest of faba beans in order to test whether high $N_2$ fixing and P efficient faba bean varieties and residue management are beneficial to subsequently grown wheat. The grain yield and grain N yields of wheat crop grown after the faba bean varieties were improved significantly compared to wheat grown after wheat. Compared to the wheat-wheat rotation, *Moti*(+) and *Obse*(+) as pre-crop variety with improved residue management significantly improved wheat grain yield by 65 and 82% demonstrating substantial beneficial effects of the improved faba bean system to the succeeding wheat. *Moti*(+) further improved the total biomass yield of rotational wheat by 112% compared to wheat(-), respectively. In addition, *Moti*(+) and *Obse*(+) significantly improved grain N yield of rotational wheat by 74% and 92% wheat – wheat rotation, respectively. The incorporated legume root, nodule and straw might have played role in improving wheat yield through N addition via BNF and straw P addition. Moreover, the faba beans might have positive effects of N sparing as shown by the lower N uptake of faba beans compared to wheat in the present study. Similar responses in grain yield of cereals to previous crops of tropical legumes were reported by Peoples and Herridge (1990) varying from 0.2 to 3.7 Mg $ha^{-1}$, corresponding to a relative increase of 16 - 353%, compared with cereal-cereal rotation. Habtegebrial and Singh (2006) have observed a 39 - 101% wheat grain yield improvement after field peas relative to wheat-wheat rotation and Habtemichial et al. (2007) recorded 36% and 66% grain and N yield advantage of faba bean wheat rotation over the wheat-wheat rotation. The positive wheat response to grain and N yield may be partially attributed to the transfer of biologically fixed $N_2$ from the precursor legume (Chalk, 1998; Habtegebrial and Singh, 2006). However, no single factor has been shown to be solely responsible for the positive effects of legumes grown in rotation with cereals. Other rotational but non N benefits including enhancement of soil microbial activity, improved soil physical and chemical properties, reduced disease and weeds incidence were reported (e.g. Peoples and Crasswell, 1992; Bagayako et al., 2000; Giller, 2001).

Wheat grain and total biomass yield after variety *Degaga* resulted in yield reduction by 9 and 3% compared to the wheat yield obtained after the local faba(-). Further, wheat grain and biomass yield obtained after this variety was not significantly different from the yield of wheat after wheat rotation. Calculation of the N budget for variety Degage in the first crop phase has shown that the N derived from soil (Table 4.3) was significantly higher and the net amount of N it adds to the soil for the wheat growth phase was lower compared to mineral N fertilizer requirement (82 kg N ha$^{-1}$) of wheat in southwest Ethiopia indicating that this variety may not be a good option in legume inclusion in the cropping system in the absence of combined mineral N application. Moreover, total biomass and grain yield of wheat obtained after wheat was 24 and 21%, lower than of wheat after the local faba bean(-), It indicates that the current farmers practice, wheat-wheat continuous cropping without mineral fertilizer, may lead to substantial wheat yield reduction if the practice is not changed.

Moreover, it was found that N uptake by the second wheat (42 kg N ha$^{-1}$) was much lower (ca. 50%) than the N uptake by the first wheat (98 kg N ha$^{-1}$) which may be related to the higher N harvest indext of the first wheat (75%) compared to the N harvest index of the faba bean varieties (49 -54%) suggesting also that wheat-wheat rotation could potentially deplete soil N.

The positive effect of legumes on rotational non-legume goes beyond an improved N nutrition (Bergerou et al., 2004). Among these effects are improved P nutrition (Jemo et al., 2006; Pypers et al., 2007; Nuruzzaman et al., 2005) and favorable microbial community in the rhizosphere (Marschner et al., 2004; Yusuf et al., 2009b). Although significant proportion of P was removed by the previously harvested grains of *Moti* (PHI = 43%) and *Obse* (PHI = 54%), our results showed that total P yield of wheat after *Moti* was 117% and after *Obse* 52% greater than that of wheat grown after wheat. Grain P yield of wheat was also 49% and 95% higher after

*Moti* and *Obse*, respectively, compared to wheat after wheat. In a pot experiment to determine the contribution of P by faba bean residue to subsequent corn using $^{33}P$ labeled legume residues, Nachimuthu et al. (2009) have shown that faba bean residues alone contributed about 10% of the total P uptake by corn as compared to 54% by inorganic P fertilizers. Further, we have observed that between 12 and 16 kg P $ha^{-1}$ could potentially remain in the soil as plant residues after removal of grains and pods while between 6 and 12 kg P $ha^{-1}$ are taken up by the second wheat which suggests a very high coefficient of utilization of P (50 – 68%). The high coefficient of utilizing P may suggest that besides P derived from the soil and plant residue there might be a strong residual effect of the P added as ORGA fertilizer in the legume crop phase. Such results may suggest that incorporation of the legume residues alone may not always lead to net P release to subsequent crops. Nuruzzaman et al. (2005) in a pot experiment where wheat was grown after wheat, faba bean, white lupin or pigeon pea concluded that rotational effects were likely related to the recycling of mobilized P through the crop residues. In the same study faba beans had the highest rotational effect (more than field pea and white lupin), but the lowest secretion of organic acids. Therefore, rotational effect of faba beans was possibly related to the recycling of mobilized P through the crop residues (Horst et al., 2001; Nuruzzaman et al., 2005). It has also been reported that P benefits to wheat are probably due to mineralization of residue derived organic P (Nuruzzaman et al., 2005).

It is understood that the use efficiency of organic N and P might not be as high as that of fertilizer N and P. So, the N balances and P content of the legume residue of the precursor faba bean varieties might not be enough to reach the same cereal yield as with mineral fertilizers. It is therefore important to see the possible benefits from a combined application of crop residue and a smaller quantity of fertilizers N and P given to the cereal grown after the faba bean. Recently, Chivenge et al. (2011) reported that maize yield responses and N use efficiency were greater following the combined application of organic residues with low quantities N fertilizers compared to the addition of either resource alone, offering potential for increasing crop productivity. From the results of the present study, faba bean variety *Moti* appears to be very suitable N and P-mobilizing legume crop for rotation in the tropical highlands where soils have low available phosphorus.

## 4.5. Conclusion

Faba bean could serve as an improved alternative to fallow particularly in areas affected by high cropland pressure, benefiting the farmers with additional income and improving or maintaining the soil fertility and in turn, increases the yields of the subsequent cereal like wheat provided that the legume residues are retained on the field. Our results showed that improvement of residue management of faba beans introduced into the wheat based cropping system that are affected by high cropland pressure and abandonment of natural fallows will have a positive effect on the yield of wheat grown in rotation through the cycling of more N and P via crop residues. This can be achieved provided that the legume residue is retained on the field and incorporated into the soil prior to the cereal mono crop. The incorporated legume root, nodule and straw might have played role in improving wheat yield through positive effects of N via BNF and straw P addition. Therefore, we argue that the N and P benefits to rotational wheat are probably due to mineralization of crop residue derived organic N and P. However, we cannot exclude the possibility that ORGA fertilizer has brought about a residual effect to wheat. Mirutse et al., (2009) reported such a residual effect of P from ORGA on teff.

The study demonstrates the prospects and importance of faba beans to be suitable alternative grain legumes for sustainable wheat based cropping systems in the humid tropical highlands of Ethiopia. Faba bean varieties that combine a reasonable grain yield with a large volume of biomass could offer a useful compromise for meeting farmers' food security concerns and improving soil fertility. However, when designing more sustainable cropping systems, farmers' preferences, perceptions and criteria for selecting different faba bean varieties for use in their cropping systems should also be assessed besides N and P benefits. Mulusew et al. (2012) have shown that plant establishment, stem strength (resistance to lodging), number of branches, seed size and marketability were among the selection criteria used by farmers.

# Chapter 5

# Climatic, edaphic or variety controls on biological $N_2$ fixation and P acquisition efficiency of faba beans

## Abstract

Environment and legume genotype constrain $N_2$ fixation, phosphorus acquisition efficiency (PAE) and hence grain and biomass production. Therefore climatic, edaphic or variety controls on $N_2$ fixation, PAE, rhizosphere phosphatase activity and the population of endophytic bacteria associated with faba beans were studied via a mesocosm experiment at two locations with a different microclimate (*Dimtu*, 2°45'16"N; 42°17'53"E at an elevation of 1790 m and *Dedo*, 7°28'48" N; 36°52'19"E at an elevation of 2160 m) in southwest Ethiopia. To separate climate, edaphic and genotype effects, we exchanged soil between the two locations and grew two faba bean varieties (*Moti* and *Obse*). Results revealed that grain and biomass yield and nodulation characteristics of faba beans were more determined by microclimate than soil or variety ($P < 0.05$). $N_2$ fixation of faba beans was determined by microclimate, soil type and variety main effects; $N_2$ fixation was higher for *Dedo* (1.2 ± 0.1 g N plant$^{-1}$) compared to *Dimtu* (0.4 ± 0.0 g N plant$^{-1}$) and variety *Moti* showed a higher $N_2$ fixation (0.9 ± 0.2 g N plant$^{-1}$) compared to *Obse* (0.6 ± 0.1 g N plant$^{-1}$). Further, highest PAE by faba beans was achieved for *Moti* grown in *Dedo* soil at location *Dedo* (99.0 ± 6.0 mg P plant$^{-1}$) and the lowest was for *Obse* grown in *Dimtu* soil at location *Dimtu* (25.0 ±3.6 mg P plant$^{-1}$) suggesting that climate controls PAE of faba beans regardless of soil type. The rhizosphere phosphatases activity was more determined by soil type than microclimate or variety effects, being the highest for the *Dedo* soil (2.1 µg g$^{-1}$ soil). Based on 16S rRNA gene sequencing, five different genera of endophytic bacteria- *Rhizobium*, *Bacillus*, *Arthrobacter*, *Staphyllococcus* and *Leifsonia* were identified from the root nodules of faba beans grown in two soil types. The host plant-endophytic bacteria associations appear not to be affected by any of the factors that we considered in the present study.

In conclusion, there is strong evidence that grain and biomass yield and nodulation characteristics of faba beans were determined more by microclimate than soil or variety effects. Soil type was more important in determining the rhizosphere phosphatases activity than microclimate or variety effects. PAE was however determined by microclimate-soil type interaction rather than soil type alone. The latter suggests that mechanism other than rhizosphere phosphatases activity might have been involved in the PAE of faba beans.

Key word: faba bean, $N_2$ fixation, P acquisition, endophytic bacteria, phosphatase

## 5.1. Introduction

Arable land resources are limited. Meeting food needs in some regions has already led to the adoption of agricultural practices that degrade the soil, and to the use of land that is marginal for crop production (Hungria and Vargas, 2000). The situation is most serious in the tropics, due to fragile soil structure, erosion, low soil organic matter content and inappropriate farming systems (Giller and Wilson, 1993). Nutrient depletion and soil acidity are two consequences of inadequate soil management. In this context, biological nitrogen fixation (BNF) and P acquisition (PAE) and utilization efficiency (PUE) are critical elements in sustainable agronomic intensification where nitrogen (N) and phosphorus (P) are the nutrients that most frequently limit crop production. Tropical climatic can constrain BNF, PAE and PUE and hence grain and biomass production.

Faba bean (*Vicia faba* L.) is of great importance in legume-cereal production systems where it is used as break crop for cereals (Amanuel et al., 2000) and has the potential to enhance N and P nutrition of cereals (Habtemichial et al., 2007; Nuruzzaman et al., 2005; Rose et al., 2010). Faba bean grows with optimal performance in good-structured clay or loam soils at temperatures between 18 and 27°C (Jensen et al., 2010; Link et al., 2010). The crop is well adapted to diverse soil types of Ethiopia where legumes are prominently used as traditional soil fertility enhancing crops in mixed cropping systems (Agegnehu et al., 2008). Besides its worldwide use for food and feed, extensive knowledge exists about its ability to symbiotically fix and add N to the soil, making additional N available and thereby enhancing and sustaining productivity (Köpke and Nemecek, 2010). Faba bean has also been reported to be a very suitable P mobilizing legume crop for rotation on soils with low available phosphorus (Nuruzzaman et al., 2005).

The N content of faba bean is determined by the relative reliance of the crop upon N fixation for growth (i.e. the proportion of crop N derived from atmospheric N, %Ndfa) and the amount of N accumulated by the crop over the growing season (Jensen et al., 2010). This also has consequences for grain and biomass yield. A wide range of environmental stress factors (e.g. water, temperature, nutrient deficits, soil pH) and management factors (plant density, weeding, cropping system, pests and diseases, etc.) determine legume biomass production and therefore the associated BNF (Zahran, 1999; Hungriaa and Vargas, 2000).

The %Ndfa at field level is determined by two important factors: the potential of legume plants to establish an effective symbiosis with $N_2$ fixing rhizobia, and the relative ability of the established symbiosis to fix N (Giller, 2001). The latter depends on the genetic potential of the rhizobia (Kihara et al., 2010), the crop and the symbiosis (Ammanuel et al., 2000; Giller, 2001). In controlled experiments, %Ndfa could be as high as 100%. However in farmers' fields, environmental and management factors frequently limit an effective legume-rhizobia symbiosis and therefore lower the potential %Ndfa and hence BNF (Unkovich et al., 2008).

With low N fertilizer inputs, the availability of soil P is often the main factor restricting N fixation. This is common in low-pH, Fe-rich soils and results in limited growth of rhizobia, restricted growth of the nodules (Kihara et al., 2010) and a reduced growth of the faba beans (Ammanuel et al., 2000). Although nodule growth was diminished at low P availability, Kouas *et al.* (2005) found that individual nodule biomass and efficiency of N fixation was not sensitive to P deficiency. However, N fixation may be reduced when plants are deficient in P. This could be explained by the effect of low P supply on the growth of the host plant, on the growth and functioning of the nodules or on the growth of both the nodule and the plant (Sa and Israel, 1991; Almeida et al., 2000).

The availability of P in the rhizosphere is influenced by soil pH and root exudates, which can directly or indirectly affect nutrient availability and microbial activity (Richardson et al., 2009; Zhang et al., 2010; Shen et al., 2011). Acidification of the rhizosphere in response to P deficiency has been demonstrated for a number of species (e.g., Hinsinger et al., 2003) and can modify the solubility of sparingly soluble inorganic P compounds and the subsequent availability of phosphate and various micronutrients in soil solution (Richardson et al., 2009). Secretion of phosphatase enzymes into the rhizosphere is a universal plant response to P deficiency (Vance et al., 2003; Richardson et al., 2005). Phosphatase activity is significantly greater in the rhizosphere than the bulk soil and is considered to be a general pathway of plants to mobilize P from organic forms. Nuruzzaman et al. (2006) studied rhizosphere phosphatase activities of legume (faba bean, field pea and white lupin) and cereal (wheat) crops. They found that phosphatase activity was detected in each species; however activities were higher in the legume species compared with wheat. Differences in phosphatase activities between crop species of cereals, legumes and oil crops (e.g., Yadav and Tarafdar, 2001) and between varieties of the same species for instance, common beans (e.g., Helal et al., 1990), cowpea and soybean (e.g., Jemo et al., 2006) have been reported. Except one report by Nuruzzaman et al. (2006), we are not aware of additional studies regarding rhizosphere phosphatase activity in faba bean.

In addition to secretion of phosphatase enzymes, many legumes form symbiotic and non-symbiotic associations with soil bacteria (Sturz et al., 1997; Zakhia et al., 2006; Kan et al., 2007; Li et al., 2008; Palaniappan et al., 2010). Faba beans form symbiotic association with *Rhizobium leguminosarum* and have been investigated in various studies (Tian et al., 2007; Tian et al., 2008; Mutch and Young, 2004). Other non-symbiotic bacterial species may live endophytically inside the nodules and do not cause any visible damage to the plants (Palaniappan et al., 2010). These non-symbiotic bacterial species can affect plant growth either in a positive or negative way. Some bacteria exert several beneficial effects on host plants and were grouped as Plant Growth Promoting Bacteria (PGPB) (Sturz et al., 1997). These bacteria have positive effects on plants through various mechanisms such as producing plant growth regulators like IAA (Indole-3-Acetic Acid) and phosphate solubility (Li et al., 2008; Sanginga and Woomer, 2009; Palaniappan et al., 2010).

Considering that the symbiotic and non-symbiotic bacteria associated with faba beans in the humid tropics of southwest Ethiopian soils have not been systematically studied and that the rhizobia - faba bean symbiosis is vital for sustainable agricultural intensification (Graham and Vance, 2000; Graham and Vance, 2003; Resh et al., 2002), we are interested in investigating the endophytic bacteria associated with faba beans grown in contrasting soils and climatological conditions. The aim of this study was therefore to assess climate, edaphic, and variety controls on BNF, P acquisition and use efficiency, rhizosphere phosphatase activity and the population of rhizobia associated with faba beans. To separate climatic and edaphic effects, we performed a mesocosm experiment in which faba bean varieties were grown at two locations with a different climate. To assess the soil effect at each of the two locations, we mutually transplanted soil between of two locations (Dimtu and Dedo sites). We hypothesized that (1) if climate is limiting, soil from a location which is marginal for faba beans in terms of climate (Dimtu) will give a better yield when transferred to a location that is suitable in terms of climate (Dedo) and soil from Dedo will give reduced yield at Dimtu; and (2) if soil is the limiting factor, soil from Dimtu will also give bad results when transferred to Dedo.

## 5.2. Materials and methods

### 5.2.1 Mesocosm field sites

A mesocosm study was carried out on farmers' fields in southwest Ethiopia at two different altitudes which are distinct in climate and soil type, namely at Dimtu (lower altitude) and Dedo (higher altitude). Soils having the same fertility level within a climate zone but differ between climate zones (Dedo: good fertility class; Dimtu: poor fertility class) were selected for this study.

**Dimtu**

Dimtu is located at 2°45'16" latitude and 42°17'53"longitude at an elevation of 1790 m above sea level. The average yearly rainfall is 1300 ± 404 mm (data for 1980-2011 from the National Meteorology Agency of Ethiopia). The average minimum and maximum temperature in 2011 was 10.0 and 29.0 °C, respectively. Top soil (0 – 30 cm depth) has a clay content of 77.3% and a silt content of 19.7%. Total organic carbonand nitrogen contents amount 1.5% and 0.2%, respectively. The Dimtu soil has an acidic pH-$H_2O$ (5.2) and shows low available P content (Bray-II = 3 mg P $kg^{-1}$ soil). This soil is classified as a Nitisol in the FAO/WRB system. The area is characterized by cereal based cropping system. The major crops are maize, teff and sorghum whereby mono-cropping of these cereals dominates in the area. Faba beans also grow here on scattered plots and around homesteads. Topographically, flat lands dominate the area with limited vegetation cover due to removal of natural vegetation because of cereal cultivation and overgrazing (Bekele et al., 2010).

**Dedo**

Dedo is located at 7°28'48" N and 36°52'19"E at an elevation of 2160 m above sea level. The average yearly rainfall is ca. 1874 ± 77 mm (data for 1975-2011 from the National Meteorology Agency of Ethiopia). The average minimum and maximum temperature in 2011 was 12.7 °C and 23.5 °C, respectively. Top soil (0 – 30 cm depth) has a clay content of 53.3%, silt content was 44.7%, total organic carbon 3% and nitrogen 0.3%, it has an acidic pH-$H_2O$ (5.6) and showed low available P (Bray-II = 10 mg P $kg^{-1}$ soil). Participatory rural appraisal (PRA) research indicated that the cropping system is characterized by cereal (wheat, teff, barley) and legume (faba bean, field peas) production. Dedo is generally characterized by optimal climate and soil conditions for growth and performance of faba beans (Bekele et al., 2010).

### 5.2.2 Experimental mesocosm design

12 pits (each 100 × 100 cm wide, 60 cm deep) in two blocks (six pits per block) were dug 50 cm apart at each location during June, 2012. At the bottom of each pit, a root mat (Ökolys, permeability of 5 liter water $hr^{-1}$ $m^{-2}$, Beaulieu International Group) was lined up to prevent root growth beyond 60 cm depth. Hence plants could not have access to nutrients below 60 cm. Top soil (0 – 30 cm) and sub soil (30 – 60 cm) was excavated from the higher altitude location, Dedo, and transferred to the first six pits at lower altitude, Dimtu, and vice versa. The next six pits received topsoil and subsoil of the native local soils (e.g., Dedo soil in Dedo pits and Dimtu soil in Dimtu pits). Some fourty days before seed sowing (June 8-10), the top and sub soils were re-installed in their respective positions in the pit of each location to allow the soil to stabilize and assume its original layer. Two faba bean varieties namely, *Moti* and *Obse* were compared, and each variety was replicated in 3 mesocosms within each climate×soil

combination. The faba bean was sown on July 18, 2012 at Dedo and on July 21, 2012 at Dimtu at a spacing of 10 cm within rows and 30 cm between rows. The two varieties were selected because they had good adaptation potential, high grain yield, high BNF, positive soil N balance and high P utilization efficiency among six faba bean varieties tested in the 2008-2009 cropping seasons at Dedo (Nebiyu et al., 2013). No N or P fertilizer was added during the course of the experiment.

### 5.2.3 Soil sampling and analyses

Composite soil samples (separately for 0-30 and 0-60 cm depth) were collected from each location for soil physico-chemical analysis. After air-drying, soil was ground and sieved (2 mm) and analyzed for pH, texture (wet sieving of the different fractions using the law of Stokes; Day, 1965), CEC and exchangeable bases (by column percolation with $NH_4$-acetate at pH 7; van Reeuwijk, 2002), available P (Bray-II P, Bray and Kurtz, 1945) and total phosphorus (Bowman, 1988). Total carbon (TC) and nitrogen (TN) were analyzed with an Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). Table 5.1 shows the soil physicochemical characteristics of the experimental sites.

### 5.2.4 Plant sampling and analyses

During late flowering, five faba bean plants were randomly selected and uprooted from the first and third rows for nodulation assessment, leaving the second (middle row) intact for grain yield and biomass measurement. Soil adhering to the roots was removed by washing with tap water. The nodules from each plant were removed, counted and the dry weight (mg plant$^{-1}$) was measured. Plants from the middle row in each mesocosm were harvested at physiological maturity and subsequently separated into grains and haulm residues (shoots and empty pods). Samples were dried at 70 °C for 48 hours and ground. Grain dry yield (GDY g plant$^{-1}$) and total biomass yield (TBY g plant$^{-1}$ = GDY + shoot (SDY g plant$^{-1}$) + empty pod (PDY g plant$^{-1}$) dry yield were calculated on dry weight basis. Nearby wheat fields at Dedo and teff (*Eragrostis tef*) at Dimtu were also sampled at the same time as reference crops for BNF measurement via the $^{15}$N natural abundance method (Peoples et al., 2009).

Table 5.1. Mean ± standard deviation of physicochemical characteristics of Nitisol mesocosm soils (Dedo: location at 2160 m a.s.l. and Dimtu: location at 1790 m a.s.l.)

|  | $pH_{H2O}$ | $pH_{KCl}$ | TC | TN | TP | Av P | Clay | Silt | Sand | CEC | Ca | Mg | Na | K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil |  |  | (%) | (%) | -------(mg P $kg^{-1}$)--- |  | --------------- (%) --------- |  |  | -----------------------($cmol_c$ $kg^{-1}$)---------------- |  |  |  |  |
| Dedo | 5.6±0.1 | 5.2±0.1 | 3.0±0.1 | 0.3±0.1 | 1156±18 | 10.7±2.5 | 53.3±3.1 | 44.7±3.1 | 2.0±0.1 | 36.5±1.4 | 12.6±0.1 | 2.3±0.1 | 0.1±0.1 | 1.3±0.2 |
| Dimtu | 5.2±0.1 | 4.7±0.1 | 1.5±0.1 | 0.2±0.1 | 662±69 | 3.0±1.7 | 77.3±0.6 | 19.7±0.6 | 3±0.0 | 29.2±0.8 | 6.5±0.0 | 2.4±0.0 | 0.4±0.3 | 1.0±0.1 |

TOC = Total C, TN = total N, TP = total P, Av P = Available P (Bray-II)

### 5.2.5 Phosphorus analysis

P concentration (mg P $g^{-1}$ DM) in shoot, grain, and pod samples was determined according to Chapman and Pratt (1961) using slight modifications described in Ryan et al. (2001). Plant samples (0.5 – 1.0 g) were ashed in porcelain crucibles for 5 hours at 550°C. The ash was dissolved in 5 mL 2N HCl and milli-Q water was added until a volume of 25 mL. The solution was filtered through Whatman No. 5 filters. P in filtrates was analyzed with an auto-analyser (Autoanalyzer 3, Bran+Luebbe, Norderstedt, Germany). P uptake (phosphorus acquisition efficiency, PAE, mg P $plant^{-1}$) in each plant part was calculated by multiplying the P concentration with the respective plant biomass (shoot, grain and pod). Total P uptake in total aboveground biomass was therefore calculated as: PAE = shoot PAE + grain PAE + pod PAE. P utilization efficiency (PUE, g grain $g^{-1}$ P) was defined as the grain yield (GDY) per unit of P accumulated in the total aboveground biomass (Rose and Wissuwa, 2012) and was given as: GDY g $plant^{-1}$/total PAE g $plant^{-1}$.

### 5.2.6 Nitrogen analysis and BNF calculation

Total nitrogen content (TN) and $\delta^{15}N$ in plant samples was analyzed with Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (20-20, SerCon, Crewe, UK). After determination of TN in the plant samples, the nitrogen yield was calculated as: Total N yield (g N $plant^{-1}$) = dry mater yield (g $plant^{-1}$) × %N/100. The percentage of plant N derived from atmospheric $N_2$ (%Ndfa), was calculated based on $^{15}N$ measurements in faba beans and reference plant (Peoples et al., 2009) as:

$\% \text{ Ndfa} = 100 \times (\delta^{15}N_{\text{reference plant}} - \delta^{15}N_{\text{legume}}) / (\delta^{15}N_{\text{reference plant}} - B \text{ value})$

Where, $\delta^{15}N_{\text{reference plant}}$ and $\delta^{15}N_{\text{legume}}$ are the whole plant $\delta^{15}N$ values of wheat or teff and faba bean, respectively. The *B* value is the $\delta^{15}N$ value of faba beans grown in sterilized vermiculite for 47 days (flowering stage) with a nutrient solution free of N in a growth chamber (Nebiyu et al., 2013). The amount of $N_2$ fixed by the legume ($N_2$ fixed, kg N $ha^{-1}$) = total N yield × (%Ndfa)/100.

### 5.2.7 Analysis of acid and alkaline phosphatase activities

Rhizosphere soil was sampled during the flowering stage of the faba beans. The activity of phosphatase in soil was analyzed within 7 days after sampling by the method of Tabatabai and Bremner (1969), as modified by Hedley et al. (1982). The analysis involved colorimetric estimation of the p-nitrophenol released by phosphatase activity after incubation of 1 g soil with 4 mL of 0.04 M sodium malate buffer (pH 6.5) and 0.1M NaOH (pH 11) at 37 °C for 1 h for acid and alkaline phosphatase, respectively. The reaction was ended by addition of 1 ml of 0.5M $CaCl_2$ and 4 ml of 0.5M NaOH. The suspension was filtered through a Whatman no. 2 filter paper and absorbance was measured spectrophotometrically at 410 nm using a UV/VIS spectrophotometer (DU-64, Beckman, USA). One unit of acid phosphatase activity was defined as the activity per gram soil, which produced 1 µmol p-nitrophenol per hour.

### 5.2.8 Symbiotic and non-symbiotic bacteria isolation

#### *5.2.8.1 Bacterial isolation*

Three plants per mesocosm from each location were sampled at the full flowering. Nodules were carefully removed from each plant and about 15-20 nodule samples were pooled from each plant per mesocosm. The nodule samples were kept in plastic vials, which were first partly filled with desiccated silica gel (Chameleon ®) on top of which a cotton lining was placed. The samples were kept in refrigerator (4 °C) until further analyses (Bala et al., 2010).

The isolation and purification of the bacteria was then performed according to Vincent (1970) using the Yeast Mannitol Agar (YMA) plates. Nodules were first sterilized to avoid the isolation of rhizobacteria from nodule surface (De Meyer et al., 2011). After four days of incubation at 28°C in aerobic conditions, visible colonies appeared and from each plate 2 to 5 colonies were selected for further analysis.

#### *5.2.8.2 Application of MALDI-TOF MS*

Matrix-Assisted Laser Desorption/Ionization (MALDI) Time-of-Flight (TOF) (MALDI-TOF) mass spectrometry (MS) of cell extracts was carried out to screen the bacterial isolates. The MALDI-TOF mass spectra of whole bacterial cell extracts with a certain mass range can be compared, allowing quick identification or characterization of microorganisms (Ryzhov and Fenselau, 2001). Here the method was used as a fast way to reduce the number of strains for further characterization by allowing recognition of identical isolates (Doan et al., 2012). The cost of this procedure is relatively low compared to the cost and the amount of work involved in sequencing the DNA samples. However, MALDI-TOF cannot discriminate for sub-species of unknown isolates as well as starins.

Bacterial cell extracts were prepared and subjected to MALDI TOF MS as described by Wieme et al. (2012). The resulting mass spectra were processed and converted into peak profiles that were clustered using Bionumerics 5.1 (Applied Maths, Belgium) (Wieme et al., 2012). In the resulting dendrogram bacteria within a cluster showing more than 80% similarity were considered to be the same species.

#### *5.2.8.3 Total DNA extraction and 16S rRNA gene PCR amplification*

DNA extracts were derived from pure bacterial colonies using the alkaline lysis method as described by Baele et al. (2000). Extractions were preserved at -20°C for later use. The 16S rRNA gene was PCR amplified using conserved general bacterial primers. Partial sequencing was performed using the primer BKL1 (Cleenwerck et al., 2007) which will cover the variable v1 to V3 region of the gene.

Finally, the partial 16S rRNA gene sequences were identified by Basic Local Alignment Search Tool (BLAST) search using the European Molecular Biology Laboratory (EMBL) database, a database that contains a large collection of nucleotide sequences to ascertain their closest related sequence.

## 5.3. Statistical analysis

All the field data were subjected to three-way (location-soil type-variety) analysis of variance using the general linear model (GLM) procedure of Statistical Analysis System (SAS) software version 9.2 (SAS, 2008). All the data were first checked for normality using the Proc univariate, option Normal procedure of SAS. Quantile-Quantile plot (Q-Q plot) on the residuals from ANOVA was taken into consideration for assessing the normality of the data. Whenever the F-test denotes significant effect due to the main effects or interactions, the least significant difference (LSD) was used to compare treatment means at $P < 0.05$. Means were presented with standard errors to indicate variation of each measurement.

## 5.4 Results

### 5.4.1 Grain and total biomass yield

The soil type and variety or their interactions did not significantly ($P > 0.05$) affect grain and biomass yield of faba beans. Grain and total biomass yield of faba beans was only significantly ($P < 0.05$) affected by the location. The highest grain (18.0 ± 1.8 g plant$^{-1}$) and biomass (38.0 ± 1.2 g plant$^{-1}$) yield was obtained at Dedo compared to the grain (3.0 ±0.6 g plant$^{-1}$) and total biomass (13.2 ± 0.4 g plant$^{-1}$) yield of faba beans obtained at Dimtu (Fig 5.1).

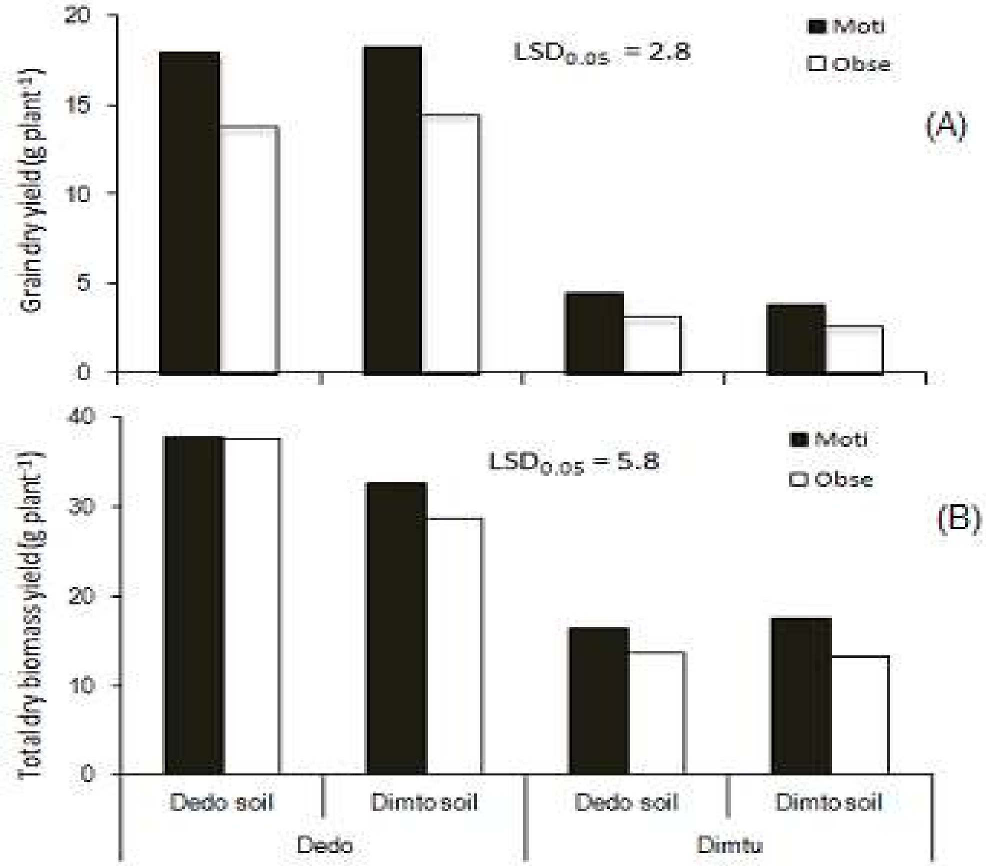

Figure 5.1. Effect of location (Dedo and Dimtu) on dry grain yield (A) and total dry biomass yield (B) of faba beans evaluated in a mesocosm study- Southwest Ethiopia, in 2012. $LSD_{0.05}$ values compare locations.

### 5.4.2 Nodule number and weight

Three way interactions of location, soil type and variety significantly ($P < 0.05$) affected the nodule number and weight per plant of faba beans. Variety *Obse* had the highest nodule number (164.5 ± 22.6) in Dedo soil in Dedo and lowest (16.2 ± 10.6) in Dedo soil in Dimtu. In addition, *Moti* showed highest nodule number (165 ± 8.7) in Dimtu soil in Dedo and lowest (44 ± 21) in Dimtu soil in Dimtu (Fig 5.2). Nodule weight was higher (350 ± 66 mg plant$^{-1}$) for variety *Obse* grown in Dedo soil at Dedo but a lower nodule weight (53 ± 30 mg plant$^{-1}$) was produced in Dedo soil transferred to Dimtu, indicating that location, hence climate, has an effect on nodule formation (Fig 5.2).

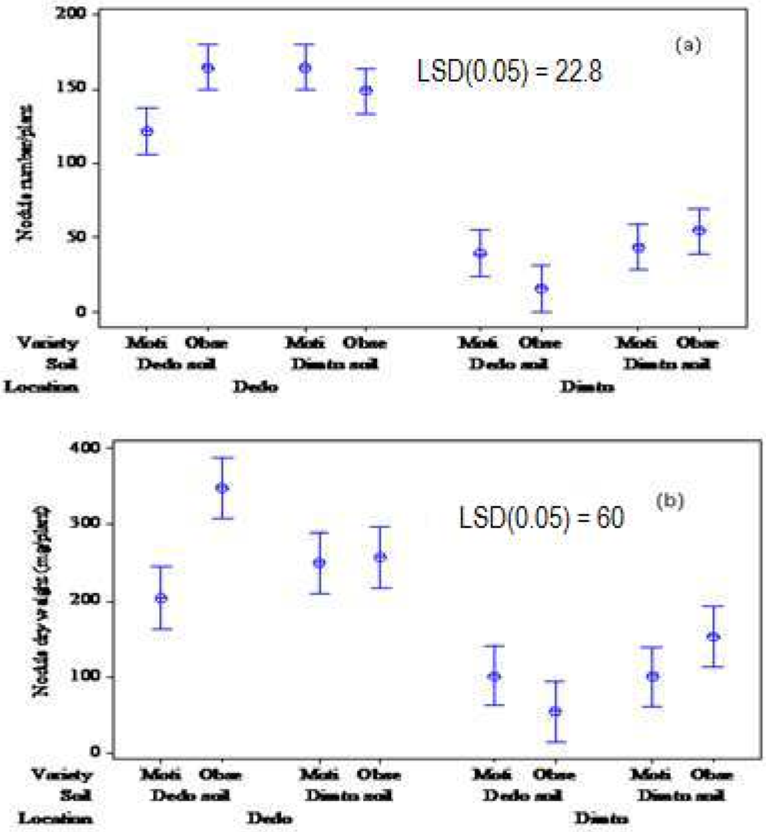

Figure 5.2. Interaction effect of location (Dedo and Dimtu), soil type (good fertility and poor fertility class) and variety (Moti and Obse) on nodule number (a) and nodule dry weight (b) of faba beans evaluated in a mesocosm study- Southwest Ethiopia, in 2012; vertical bars represent standard errors. $LSD_{0.05}$ values compare three way interactions.

### 5.4.3 %Ndfa and $N_2$ fixed

Location, soil type and variety had highly significant ($P < 0.0001$) main effects on %Ndfa (Table 5.2). %Ndfa was higher at Dedo (81.3 ± 4.0%) and Dedo soil (80.5 ± 5.3%). Variety *Moti* had significantly higher %Ndfa (85.5 ± 4.3%) compared to *Obse* irrespective of the location and soil type it grew on. No significant ($P > 0.05$) interaction between the factors was noticed for %Ndfa. $N_2$ fixed (g N plant$^{-1}$) was significantly affected by location ($P < 0.0001$) and variety ($P < 0.05$). Similarly, N fixed was higher for Dedo (1.2 ± 0.1) compared to Dimtu (0.4 ± 0.0) and variety *Moti* showed a higher $N_2$ fixed (0.9 ± 0.2 g N plant$^{-1}$) compared to *Obse* (0.6 ± 0.1 g N plant$^{-1}$). Here also, the interaction between factors was not significant ($P > 0.05$) suggesting that main effects were more important than interactions between factors. Concerning the grain and total N yield (g plant$^{-1}$) of faba beans, only the location had a highly significant effect ($P < 0.0001$) with both grain (0.91 ± 0.1 g plant$^{-1}$) and total N yields (1.4 ± 0.1 g plant$^{-1}$) were higher at Dedo compared to Dimtu. There was no significant ($P > 0.05$) interactive effect between the factors on the N yield of faba beans.

Table 5.2. Main effect of location, soil type and variety on %Ndfa, $N_2$ fixed and grain and total N yield of faba beans evaluated in a mesocosm study- Southwest Ethiopia, in 2012. Values are mean ± SE; means followed with different letters in a column are significantly different at $P < 0.05$.

| Location | %Ndfa | $N_2$ fixed (g plant$^{-1}$) | Grain N yield (g plant$^{-1}$) | Total N yield (g plant$^{-1}$) |
|---|---|---|---|---|
| Dedo | 81.3 ± 4.0a | 1.2 ± 0.1a | 0.91 ± 0.1a | 1.4 ± 0.1a |
| Dimtu | 70.3 ± 4.6b | 0.4 ± 0.0b | 0.20 ± 0.0b | 0.5 ±0.0b |
| Soil type | | | | |
| Dedo soil | 80.5 ± 5.3a | 0.8 ± 0.2a | 0.55 ± 0.1a | 0.99 ±0.2a |
| Dimtu soil | 71.0 ± 3.1b | 0.6 ± 0.1a | 0.56 ± 0.1a | 0.88 ±0.1a |
| Variety | | | | |
| *Moti* | 85.5 ± 4.3a | 0.9 ± 0.2a | 0.62 ± 0.1a | 1.0 ±0.1a |
| *Obse* | 66.1 ± 2.5b | 0.6 ± 0.1b | 0.49 ± 0.1a | 0.8 ±0.2a |
| $LSD_{0.05}$ | 9.2 | 71.2 | 0.2 | 0.2 |

### 5.4.4 Phosphorus acquisition (PAE) and utilization (PUE) efficiency

Shoot P concentration (mg P $g^{-1}$ DM) was significantly affected by soil type ($P < 0.05$) and location-variety interaction ($P < 0.05$). It was higher (1.6 ±0.1 mg P $g^{-1}$ dry matter) for plants grown on Dimtu soil compared to those grown on Dedo soil (1.2 ±0.1 mg P $g^{-1}$ dry matter) (Fig 5.3). Further, variety Obse showed highest (1.6 ±0.1 mg P $g^{-1}$ dry matter) shoot P concentration at Dedo and lowest (1.2 ±0.1 mg P $g^{-1}$ dry matter) at Dimtu while variety Moti demonstrated highest (1.6 ±0.2 mg P $g^{-1}$ dry matter) at Dimtu and lowest (1.3 ±0.2 mg P $g^{-1}$ dry matter) at Dedo indicating the presence of variety by location interaction.

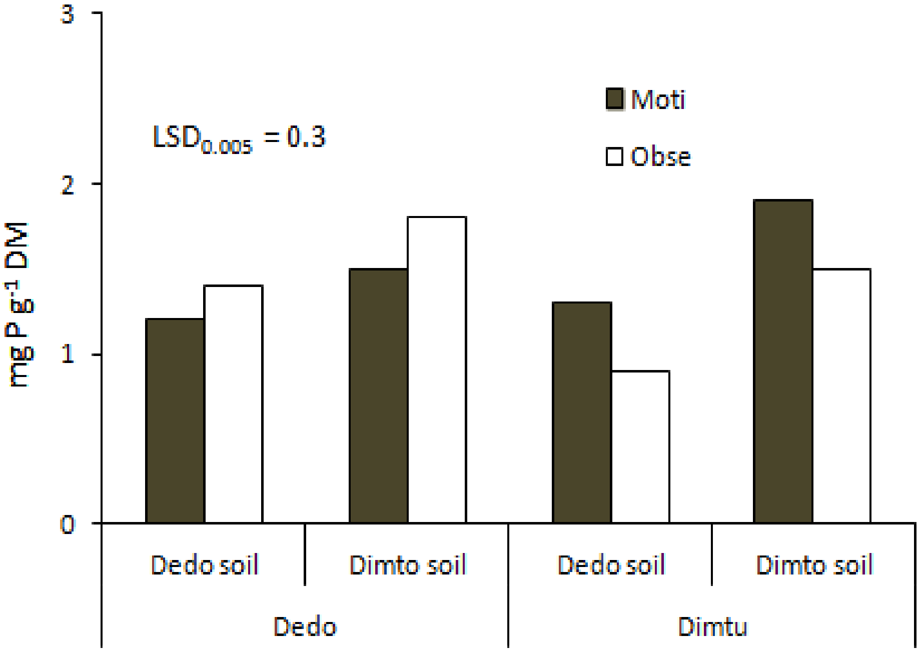

Figure 5.3. Shoot P concentration (mg P $g^{-1}$ DM) of faba bean varieties as affected by location (Dedo and Dimtu) and soil type interaction on two faba bean varieties (Moti and Obse) evaluated in a mesocosm study- Southwest Ethiopia, in 2012. $LSD_{0.05}$ values compare the locations-soil type interactions.

The interaction effect between location and soil type was significant for PAE ($P < 0.05$). The highest PAE by faba beans was achieved for *Moti* in Dedo soil at Dedo (99.0 ± 6.0 mg P plant$^{-1}$) and the lowest was for *Obse* in Dimtu soil at Dimtu (25.0 ± 3.6 mg P plant$^{-1}$) (Fig 5.4) indicating that the faba bean has a higher PAE when grown the higher altitude climate. No significant three way interaction ($P > 0.05$) between factors was observed on PAE. Further, there was no significant ($P > 0.05$) effect of the factors (location, soil type or variety) or their respective interactions on PUE (Fig 5.4).

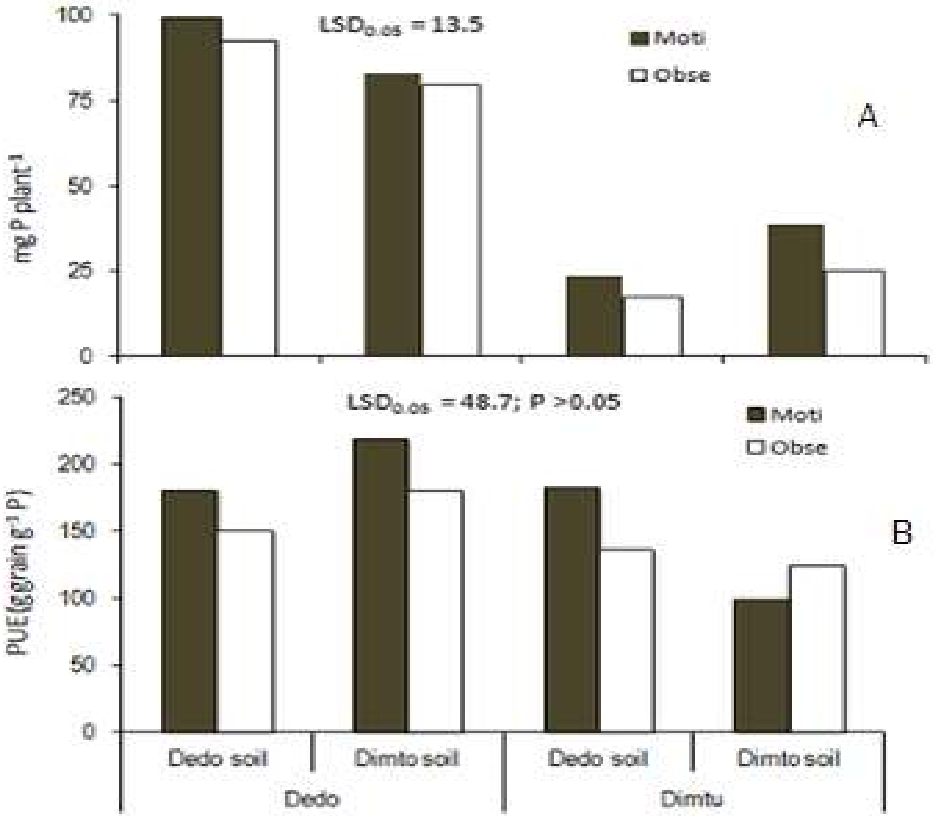

Figure 5.4. P acquisition (PAE, mg P plant$^{-1}$) (A) and utilization efficiency (B) of faba bean varieties (Moti and Obse) as affected by location and soil type interaction evaluated in a mesocosm study- Southwest Ethiopia, in 2012. $LSD_{0.05}$ values compare the location-soil type interactions.

### 5.4.5 Phosphatase activity

Both acid and alkaline phosphatase activities (µg $g^{-1}$ soil) were measured in the rhizosphere of faba bean plants. All the tested soil samples from each mesocosm were positive for phosphatase activity. However, only the soil types had a significant effect ($P < 0.0001$) for both acid and alkaline phosphatase activities. The Dedo soil showed significantly higher concentration of acid phosphatase (on average 2.1 µg $g^{-1}$ soil) and alkaline phosphatase (on average 2.0 µg $g^{-1}$ soil) compared to the Dimtu soil (Fig 5.5).

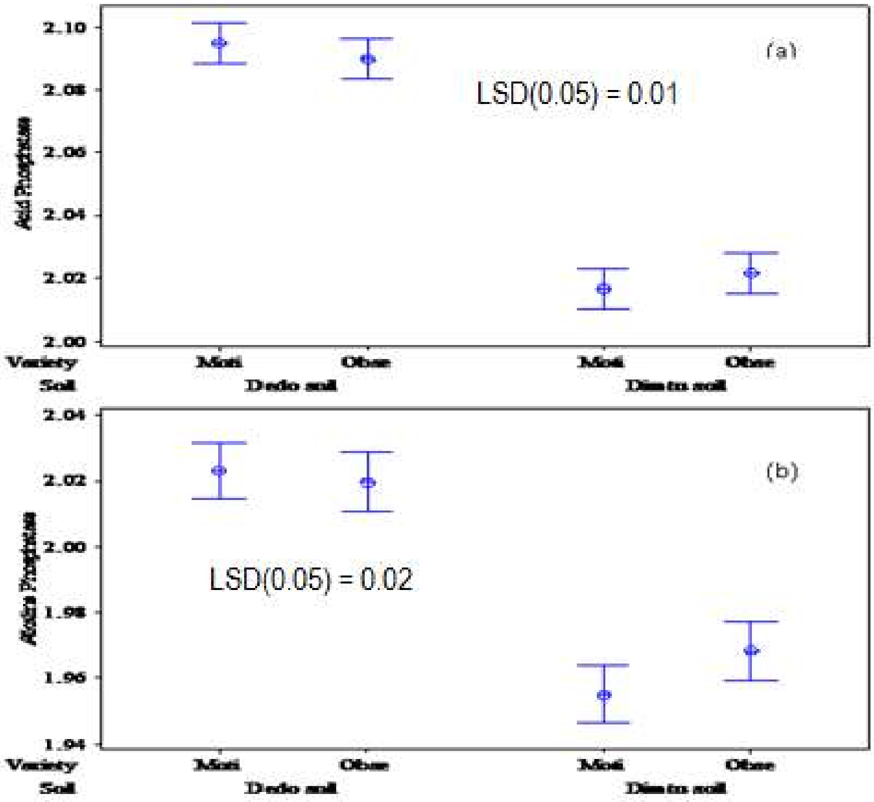

Figure 5.5. Effect of soil type on rhizosphere acid phosphatase (a) and alkaline phosphatase (b) activities of faba beans evaluated in a mesocosm study- Southwest Ethiopia, in 2012. Vertical bars represent standard errors. $LSD_{0.05}$ values compare the soil types. One unit of acid phosphatase activity is defined as the activity per gram soil, which produced 1 µmol p-nitrophenol per hour. $LSD_{0.05}$ values compare the soil types.

### 5.4.6 Symbiotic and non-symbiotic bacteria

Several bacterial species (rhizobia and endophytic bacteria) were isolated from faba bean root nodules. In most cases, the endophytic bacteria coexisted with the symbiotic *Rhizobium* strains in the same nodules. A dendrogram was constructed based on comparisons of the mass spectra obtained with MALDI-TOF mass spectrometry and isolates were divided into nine distinct clusters (data not shown) at 80% similarity. One sample of each cluster along with some samples which did not fit in cluster was further subjected to identification by partial sequencing of the 16S rRNA gene. A total of five bacterial genera (Table 5.3) were distinguished. Both N fixing and non-N fixing bacteria were isolated. *Rhizobium leguminosarum* was equally found associated with root nodules in both soils and locations while, *Rhizobium etli* was only detected in Dimtu soil at Dimtu. Among the non-N fixing bacteria, *Leifsonia spp* was only found in Dedo soil at Dedo and *Bacillus, Staphyllococcus* and *Arthrobacter spps* were found mainly in Dimtu soil regardless of the location.

Table 5.3. Rhizobia and other endophytic bacteria associated with two faba bean varieties grown at two different soil types and climatic conditions in Southwet Ethiopia. First column shows the isolate number (where the first number refers to the location, the letter refers to the soil type), second column is the associated cluster number established by MALDI-TOF and the third column represents the names of the bacterial species.

| **Isolate** | **Maldi Cluster** | **BLAST identification** | Location | Soil | Variety |
|---|---|---|---|---|---|
| 1A061t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dedo | *Obse* |
| 1B012t3 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B012t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B013t3 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B023t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 1B023t2 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 1B041t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 1B041t2 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 1B051t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B051t2 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B051t3 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B062t1 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 1B062t2 | 1 | *Rhizobium leguminosarum* | Dedo | Dimtu | *Obse* |
| 2A013t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A042t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2A051t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A051t2 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A052t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A052t2 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A053t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2A062t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2A062t2 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2A063t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2B032t1 | 1 | *Rhizobium leguminosarum* | Dimtu | Dimtu | *Moti* |
| 2B042t2 | 1 | *Rhizobium leguminosarum* | Dimtu | Dimtu | *Obse* |
| 2A041t1 | 2 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2A041t2 | 2 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 1A052t2 | 3 | *Leifsonia sp.* | Dedo | Dedo | *Moti* |
| 1A052t3 | 3 | *Leifsonia sp.* | Dedo | Dedo | *Moti* |
| 2B012t1 | 4 | *Bacillus sp.* | Dimtu | Dimtu | *Moti* |
| 2B042t1 | 4 | *Bacillus sp.* | Dimtu | Dimtu | *Obse* |
| 2B043t2 | 4 | *Bacillus sp.* | Dimtu | Dimtu | *Obse* |
| 2B043t3 | 4 | *Bacillus sp.* | Dimtu | Dimtu | *Obse* |

Table 3. continued,

| Isolate | Maldi Cluster | BLAST identification | Location | Soil | Variety |
|---|---|---|---|---|---|
| 1B033t1 | 6 | *Staphyllococcus sp.* | Dedo | Dimtu | *Moti* |
| 2B031t1 | 6 | *Staphyllococcus sp.* | Dimtu | Dimtu | *Moti* |
| 2B031t2 | 6 | *Staphyllococcus sp.* | Dimtu | Dimtu | *Moti* |
| 2B031t3 | 6 | *Staphyllococcus sp.* | Dimtu | Dimtu | *Moti* |
| 2B031t4 | 6 | *Staphyllococcus sp.* | Dimtu | Dimtu | *Moti* |
| 2B031t5 | 6 | *Staphyllococcus sp.* | Dimtu | Dimtu | *Moti* |
| 2B033t1 | 7 | *Bacillus spp.* | Dimtu | Dimtu | *Moti* |
| 2B033t2 | 7 | *Bacillus spp.* | Dimtu | Dimtu | *Moti* |
| 2B033t3 | 7 | *Bacillus spp.* | Dimtu | Dimtu | *Moti* |
| 2B041t2 | 7 | *Bacillus spp.* | Dimtu | Dimtu | *Obse* |
| 2B051t1 | 7 | *Bacillus spp.* | Dimtu | Dimtu | *Moti* |
| 1B033t2 | 8 | *Staphyllococcus sp.* | Dedo | Dimtu | *Moti* |
| 1B033t3 | 8 | *Staphyllococcus sp.* | Dedo | Dimtu | *Moti* |
| 2A063t1 | 9 | *Rhizobium leguminosarum* | Dimtu | Dedo | *Obse* |
| 2B023t2 | 9 | *Rhizobium leguminosarum* | Dimtu | Dimtu | *Obse* |
| 1A052t1 | / | *Rhizobium leguminosarum* | Dedo | Dedo | *Moti* |
| 1B012t2 | / | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 1B013t1 | / | *Rhizobium leguminosarum* | Dedo | Dimtu | *Moti* |
| 2A012t2 | / | *Bacillus spp.* | Dimtu | Dedo | *Moti* |
| 2A013t2 | / | *Rhizobium leguminosarum* | Dimtu | Dedo | *Moti* |
| 2B022t1 | / | *Rhizobium etli / pisi* | Dimtu | Dimtu | *Obse* |
| 2B023t1 | / | *Arthrobacter sp.* | Dimtu | Dimtu | *Obse* |
| 2B043t1 | / | *Bacillus spp.* | Dimtu | Dimtu | *Obse* |

## 5.5 Discussion

### Grain and total biomass yield

In the present study, grain and biomass yields of faba beans were a function of location, indicating that there was a differential response of the test varieties to the growing conditions at the two locations. Studies conducted by Flores et al. (1996), Mussa et al. (2004) and Mulusew et al. (2008) confirmed a high variety-environment interaction in faba bean grain yield. In most of these studies, environmental effects accounted for the largest part of the total yield variation and the contribution by varieties was less. As well in our study, location (climate) was found to have a greater influence than soil or variety in determining grain and biomass yield of faba beans. The two varieties performed very poor with regard to grain and biomass production at Dimtu regardless of the soil type used. Average grain yield, for instance, at Dimtu was four times lower than that obtained at Dedo indicating that location (climate) had a profound influence on faba bean growth and yield formation. These large yield differences between the locations might be due to differences in temperature variations of the respective locations (Fig 5.6). Rainfall distribution seems more or less similar. According to Patrick and Stoddard (2010), temperature has an effect on flowering, flower retention and seed formation which are key processes determining yield. For the onset of flowering, an optimum temperature of 22–23 °C is required and flowers may abscise from the plant because of heat stresses above the optimum (Patrick and Stoddard, 2010; Turpin et al., 2002). Link et al. (2010) further explained that the growing seasons should have little or no excessive heat and the optimum temperatures for production should range from 18 to 27 °C. We found that plants grown at Dimtu came to flowering but did not set the required pods. This indicated that the temperature experienced by plants at Dimtu in September (flowering period) (Fig 5.6) was above the optimum for yield and biomass formation.

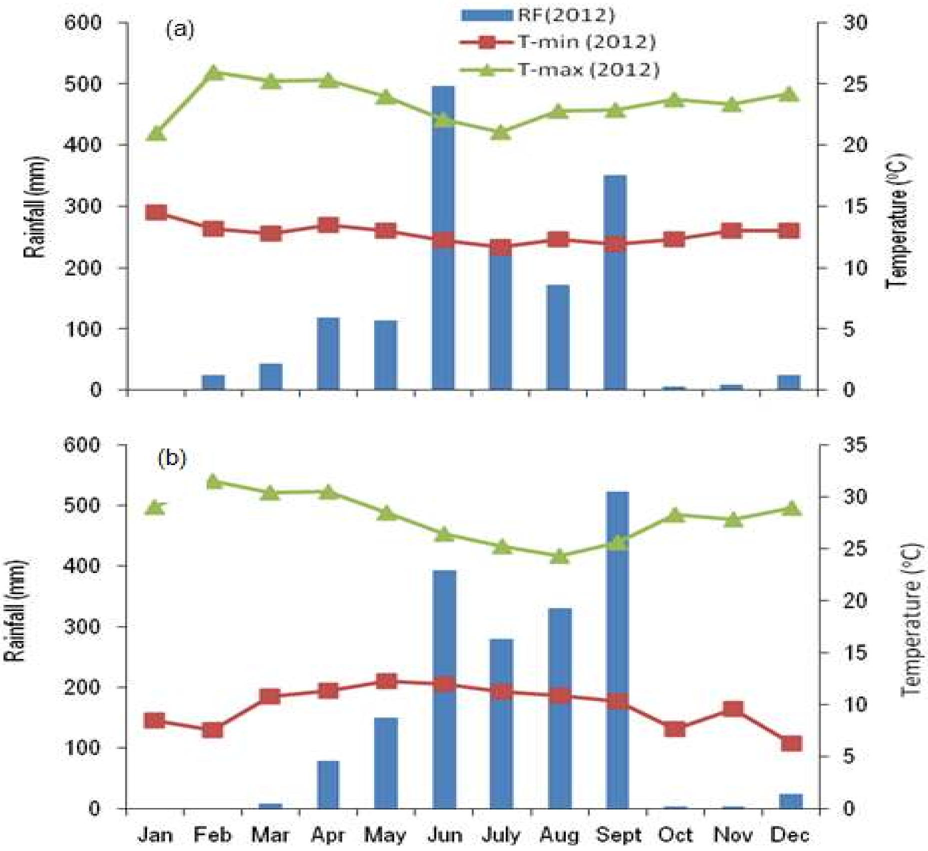

Figure 5.6. Total rainfall (RF), minimum (T-min) and maximum temperatures (T-max) of Dedo (a) and Dimtu (b) for the year 2012. Faba beans in the study area are sown in July, start flowering in September and are harvested in November.

### Nodule number and weight

In the present study, nodule formation and nodule development were evaluated using the number of nodules and the nodule weight. The average values of nodule number and weight per plant were four times higher for the faba beans grown at the higher altitude, Dedo, where temperatures and water supply were optimum compared to the lower altitude, Dimtu. This could be further explained by the fact that the faba bean plants grown at Dimtu on soils derived from Dedo gave significantly similar nodule number and weight compared with plants grown on

Dimtu soil at Dimtu suggesting that climate (temperature) was the limiting factor for the nodule growth and development in the faba bean-rhizobia symbiosis via influencing the physiological state of the plants. In addition, the low number and weight of nodules at Dimtu could be related to the P status of the soil where plant available P was very low (Table 5.1). A nutrient solution study by Olivera et al. (2008) further showed that nodule number and weight were increased due to P supply suggesting that under P deficiency, nodulation and nodule growth were impaired to a greater extent.

Faba beans form a symbiotic relationship with the soil bacteria *Rhizobium leguminosarum* and most cultivated soils contain large populations of indigenous rhizobia for faba bean (Jensen et al., 2010). However, the formation and development of a functioning symbiosis between the legume and rhizobia is dependent upon many environmental factors and management practices (Jensen et al., 2010). In the rhizobium-legume symbiosis, the process of rhizobium infection is also strongly related to the physiological state of the host plant. Nodule formation and development requires high energy (ATP), which makes P the main limiting factor. With low N fertilizer inputs, the availability of soil P is the main factor restricting nodule formation. This is common in low pH soils and results in a limited growth of rhizobia, hence restricted growth of the nodules (Leidi and Rodríguez-Navarro, 2000; Kihara et al., 2010; Kouas et al., 2005). Therefore, a competitive and persistent rhizobial strain might not express its full capacity for nitrogen fixation if limiting factors (e.g., unfavorable soil pH, nutrient deficiency, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, etc.) impose limitations on the vigor of the host legume (Peoples et al., 1995; Zahran, 1999).

### %Ndfa and $N_2$ fixed

N derived from the atmosphere (%Ndfa) is dependent on legume genotype, crop growth and plant-available N in the soil (Jensen et al., 2010), which are linked to location, soil type and variety. Our data showed that %Ndfa of faba beans was determined by variety and location. Furthermore, Unkovich and Pate (2000) reported that dry matter production potential of a plant (crop growth) is the driving factor behind N fixation when there is an effective legume-rhizobium symbiosis. This may be correct from the perspective that growth creates a demand for N (Pate and Layzell, 1990). At Dedo, plant growth performance and total biomass production was significantly higher compared to Dimtu, which supports the hypothesis that plant growth also drives N fixation. Variety *Moti* was able to establish a higher %Ndfa and therefore BNF compared to *Obse* regardless of soil type and growing environment, which further suggested that genotype had an effect on %Ndfa and BNF. The higher amount of N fixed due to effect of location (Dedo) and genotype (*Moti*) might be due to the increased biomass yield and %Ndfa due to the favorable climate at Dedo and the genetic makeup of *Moti* to establish an effective plant-rhizobium symbiosis. Peoples et al. (2009) also reported that the mount of N fixed is generally controlled by faba bean growth and variety.

### Phosphorus acquisition (PAE) and utilization (PUE) efficiency

Enhancing P efficiency by crops can be achieved through improving PAE and/or PUE (Manske et al., 2001; Shnoy and Kalagudi, 2005; Parentoni and Jùnior, 2008). However, PAE and PUE could vary with crop species and environmental conditions (Wang et al., 2010). In our study, PAE of faba beans showed a significant interaction with location and soil type. Enhanced PAE was pronounced at Dedo on the two soil types. PAE was however significantly lower at Dimtu. At Dimtu, the soil derived from Dedo resulted in lower PAE. It was therefore evident that the climate at the higher altitude was more favorable for PAE of faba beans regardless of the soil types suggesting that climate controls PAE of faba beans. The significantly lower plant biomass recorded at Dimtu might have contributed to the lower PAE, suggesting also here that location (climate) influenced PAE via its effect on biomass production.

### Phosphatase activity

Root-induced chemical and biological changes in the rhizosphere play a vital role in enhancing the bioavailability of soil P (Hinsinger, 2001), such as secretion of phosphatases to mobilize sparingly soluble mineral P and organic P sources (Richardson et al., 2009; Zhang et al., 2010; Shen et al., 2011). Our data clearly showed that the soil factor was more important in determining the rhizosphere phosphatases activity than location or variety. Both acid and alkaline phosphatase activities were significantly greater in the Dedo soil regardless of the location and faba bean varieties. Soil properties have been reported to have a great influence on the phosphatase activities (Li and Sarah, 2003). The main influence is caused by soil organic carbon (Feller et al., 1994; Acosta-Martínez et al., 2007; Li and Sarah, 2003). This may probably be due to the fact that organic carbon plays a key role to physically stabilize the enzyme (Acosta-Martínez et al., 2007). Hence, the higher phosphatase activity in Dedo soils may be explained by the higher organic C content.

Secretion of acid phosphatase is considered to be an adaptive mechanism for plants growing in P limited environments; therefore its secretion might be stimulated by low plant P status (Nuruzzaman et al., 2006; Vance et al., 2003). Nuruzzaman et al. (2006) also reported a higher acid phosphatase activity in zero P treatments of faba bean and white lupin compared to the P (15 kg P ha$^{-1}$) treatment in a greenhouse experiment, which suggested that P deficiency triggers secretion of phosphatase enzymes. The fact that we observed the lowest phosphatase activities in the Dimtu soil that is very low in available P (Bray-II P = 3.0 mg P kg$^{-1}$, Table 1) relative to the Dedo soil (10.7 mg P kg$^{-1}$) pointed to the fact that it is not the level of P-deficiency that explained the differences in phosphatase activities, but rather the higher C contents in Dedo through its protective effects on the enzymes.

Our data did not support the suggestion of Johnson et al. (1996) that the exuded phosphatase enzymes into the rhizosphere increases P availability and thus increase plant PAE. PAE was however more determined by the location-soil type interaction rather than soil type or variety alone hence we have no evidence that the phosphatase played a role in increasing the PAE. Our data rather showed that the secretion of phosphatase does not bring the PAE of the low-P location to the same level as at the high-P location suggesting that other possible mechanisms (e.g., mycorhizal association or P solubilization by rhizobacteria) might have involved in the control of PAE of faba beans.

### Rhizobia and other endophytic bacteria

Symbiotic and non-symbiotic endophytic bacteria have been isolated from the root nodules of a wide range of legumes (Zakhia et al., 2006; Kan et al., 2007; Li et al., 2008; Palaniappan et al., 2010; Wang et al., 2013). We found both symbiotic and non-symbiotic endophytic bacterial strains in association with root nodules of faba beans grown in two soil types. Considering 16S rRNA gene sequencing results, the strains mainly belong to five different genera: *Rhizobium*, *Bacillus*, *Arthrobacter*, *Staphylococcus* and *Leifsonia*.

Concerning the N fixing bacteria*, Rhizobium leguminosarum* was equally found in both areas and in both soils, while *Rhizobium etli/pisi* was only found once in the Dimtu soil at Dimtu area. The correct name of the latter one was inconclusive because of the short 16S rRNA. In a study of Tian et al. (2008), both *Rhizobium leguminosarum* and *Rhizobium etli/pisi* species were isolated from nodules originating from faba beans and the biovar was described as v*iciae* (*Rhizobium leguminosarum* bv. v*iciae*) as the most common bacterium in symbiosis with faba beans. To verify the types of biovars isolated from Dimtu and Dedo soils, further analysis, such as sequencing of the nod genes, is required. *Rhizobium etli/pisi* is described in a study of Zahran (2001) as a more sensitive strain in terms of soil pH (low or high) and temperatures. Bearing in mind that Dimtu has a lower soil pH, we can assume that this is the case why *Rhizobium etli/pisi* was isolated from Dimtu.

Previously, symbiotic and non-symbiotic endophytic bacteria have been isolated from crops such as peanut (Wang et al., 2013), *Lespedeza* spp (Palaniappan et al., (2010), *Acropora solitaryensis* (Liu et al., 2012) and herbaceous legumes (Kan et al., 2007). To the best of our knowledge, very little published data (e.g., Kan et al., 2007) is available regarding endophytic bacteria associated with faba beans except characterization and genetic diversity studies of *Rhizobium leguminosarum* (e.g., Mutch et al., 2003; Mutch and Young, 2004; Tian et al., 2007; Tian et al., 2008).

Populations of endophytic bacteria of diverse genera and species are not related to rhizobial symbiotic nitrogen fixing bacteria (Zakhia et al., 2006; Li et al., 2008). The genera

*Bacillus, Arthrobacter, Staphyllococcus* and *Leifsonia* are not involved in the symbiotic fixation of nitrogen but can be found in association with nitrogen fixing bacteria in nodules. However, these non-rhizobial endophytes were reported to improve plant growth and nodulation when co-inoculated with *Rhizobium* spp., compared to inoculation with *Rhizobium* alone (Bai et al., 2002; Rajendran et al., 2008). Endophytic bacteria in soybean and cowpea were also found to promote plant growth by producing IAA (Indole-3-Acetic Acid) and by phosphate solubization (Li et al., 2008; Palaniappan et al., 2010). Further study, however, is required to fully understand the interactions between endophytic bacteria, symbiotic bacteria, and host plant on plant growth, nodulation, and symbiotic performance in agro-ecosystems.

## 5.6 Conclusion

Our hypothesis that if climate is limiting, soil from a location which is marginal in terms of climate (Dimtu) for faba beans gives better performance at a location that is suitable in terms of climate (Dedo) was proved right suggesting that climate was the limiting factor. But the second hypothesis that if soil is the limiting factor, soil from Dimtu gives bad results when transferred to Dedo was proved wrong suggesting that soil was not the limiting factor. However, rhizosphere phosphatase activity was dependent on the soil factor. There is strong evidence that grain and biomass yield and nodulation characteristics of faba beans were determined more by the effect location than the soil or variety effect. This was explained by the fact that soil from the lower altitude with relatively low levels of total N, organic carbon, available P and soil pH when transplanted to the higher altitude showed significantly similar performance with the soil from the higher altitude having relatively high levels of total N, organic carbon, available P and soil pH. The %Ndfa and $N_2$ fixed of faba beans was however determined by location, soil type and variety main effects. It was moreover evident that soil type was more important in determining the rhizosphere phosphatases activity than location or variety effects but soil type alone did not affect PAE but the location-soil type interaction was important which highlights that another mechanism other than rhizosphere phosphatases activity might have been involved in the PAE of faba beans.

We have further shown that both symbiotic and non-symbiotic endophytic bacterial strains were found in association with root nodules of faba beans grown in two soil types. Application of MALDI-TOF profiles resulted in nine distinct clusters. Considering 16S rRNA gene sequencing results, five different genera were distinguished: *Rhizobium*, *Bacillus*, *Arthrobacter*, *Staphyllococcus* and *Leifsonia*. Further investigation on the role of these entophytic bacteria on the growth, $N_2$ fixation and P nutrition of faba beans is suggested.

# Chapter 6

# General discussion and future perspectives

## 6.1. General discussion

The abandonment of natural fallows combined with low N and P fertilizer use in cereal-based agro-ecosystems of the southwestern Ethiopian highlands led to the nutrient mining and decline in soil fertility, resulting in decreased productivity. Low phosphorus availability is particularly a principal constraint to crop production in highly weathered soils of the tropics. Introduction of dual-purpose grain legumes with high $N_2$ fixation and P use efficiency is therefore a sustainable and cost-effective way of improving both protein consumption and soil fertility in cereal-based agro-ecosystems (Rose et al., 2011; Rose and Wissuwa, 2012). Under low nutrient supply and purchasing power, integration of legume crop species in traditional subsistence cropping systems is the preferred complementary strategy to increase soil fertility (Belane and Dakora, 2010). Among legumes, faba bean is of great importance in legume-cereal rotations where it has the potential to enhance N and P nutrition of cereals through its ability to fix atmospheric nitrogen (Amanuel et al., 2000), and to mobilize and recycle the sparingly available soil P (Nuruzzaman et al., 2005; Rose et al., 2010).

Faba bean is grown worldwide in cropping systems as a grain (pulse) and green-manure legume. Faba bean contributes to the sustainability of cropping systems via: 1) its ability to contribute N to the system via BNF, 2) diversification of systems leading to decreased disease, pest and weed build-up and potentially increased biodiversity, 3) reduced fossil energy consumption in plant production, and 4) providing food and feed rich in protein.

Series of experiments were undertaken to determine the agronomic performance in terms of grain and biomass yield, BNF, PUE and N balance of faba beans. Subsequently the effect of crop residues of selected faba bean varieties on agronomic performance of wheat was investigated. Finally a follow up experiment has been performed to determine whether climate, edaphic or variety conditions control agronomic performance, BNF and PUE of faba beans. In the following sections, the most important results are synthesized and general conclusions and recommendations are drawn. Future research perspectives are also highlighted.

### 6.1.1. *B*-value determination to quantify biological $N_2$ fixation

Precise determination of the biologically fixed N by legumes is essential to determine the role of legume crops in the improvement of agro-ecosystems and the maintenance of soil N stocks (López-Bellido et al., 2006). The central problem in the quantification of the amount of biologically fixed N is the selection of the appropriate method. The natural abundance of $^{15}N$ ($\delta^{15}N$) can be used to assess BNF in field conditions without the additional cost and effort of applying $^{15}N$-enriched fertilizer. However, one important potential problem with the $^{15}N$ natural abundance technique is the requirement for a so-called *B-value* (Unkovich and Pate, 2000). The conventional estimates of *B-values* are often biased due to at least two reasons. First, they are calculated based on aerial tissues because of ease of sampling and such sampling protocols do not take into account the non-uniform distribution of $^{15}N$ between roots, nodules and shoot tissue (Boddey et al., 2000). Second, in order to obtain the true *B-value* an additional adjustment for seed N at sowing should be considered. The significant impact of seed size has often been ignored in most studies of *B-value* and BNF determination. Seed N can make up a significant proportion of total N accumulated by the crop, especially for large seeded legumes with high seed N contents like faba beans (Lopez-Belido et al., 2010). Many authors, however, use literature *B-values* to quantify %Ndfa, BNF and soil N balances.

Therefore, we have tested the importance of considering the non-uniform distribution of $^{15}$N both in the above and below ground biomass, the need for an additional adjustment for seed N and seed $^{15}$N at sowing and quantified the effect of faba bean variety on *B-values* and hence BNF estimates. Our data showed that the *B-values* were considerably different between varieties (+0.5 ± 0.4 to -1.9 ± 1.4‰) when the whole plant and seed N corrected value is used. Correcting for seed N and calculating on whole plant basis was the most correct and unbiased *B-value* calculation approach.

In the course of this study, however, we did not undertake a reliability test during the cultivation of faba beans under hydroponic conditions to assess whether all seed N has been used by the plant, or not. Lopez-Belido et al. (2010) however, showed that total N content of the plant with respect to the seed was 3 mg N lower than the original seed N content representing about 13% of the total seed N in faba bean. This may suggest that the whole original seed N might have not been utilized by the faba bean plants. Our report that N contributed by the seed at sowing to whole plant N at flowering was in the range from 34% (*CS-20DK*) to 61% could therefore be overestimated by at a maximum of 13%.

In this thesis, detailed *B-values* for faba beans have been reported, which can be used by other researchers. However, when applying the $^{15}$N natural abundance technique to compare BNF of legume accessions, we recommend determining a *B-value* for each accession. For legumes with large seeds such as faba beans, it is moreover essential to account for seed N contribution to total plant N along with some sensitivity tests to confirm that the entire seed N is indeed utilized by the growing plant when determining the *B-value*.

### 6.1.2. Crop P-efficiency and responses to P-deficiency

#### *6.1.2.1. P-efficiency*

Phosphorus efficient crops play a major role in increasing crop yields due to shortage of inorganic P fertilizer resources, limited land and water resources, and increasing environmental concerns (Wang et al., 2010). Phosphorus efficiency, which is the ability of a crop to produce high yield under P limiting condition can be attained through improved P uptake efficiency (the ability to take more P from the soil under P limiting condition) and/or through improved P utilization efficiency (the ability to produce higher dry matter yield per unit of P taken up). Thus, P-efficient crops produce reasonably high yield in low P soils through either ways and thus can reduce mineral P fertilizer input requirement in agricultural production.

On the other hand, P utilization efficient cultivars produce high yield per unit of absorbed P under P deficient conditions, since they have low internal P demand for normal metabolic activities and growth and hence have low requirement for mineral P fertilizer inputs to produce reasonably high yield. Moreover, they remove less P from soil during growth and therefore the quantity of P removed along with the harvestable parts of the crop would obviously be less, consequently reducing the quantity of mineral P fertilizer inputs required for maintenance fertilization (Balemi and Negisho, 2012). In view of these, we have demonstrated that the differences in grain yield and biomass production was attributed to the differences in PUE of genotypes. The faba bean varieties have shown much less difference in P uptake at low-P conditions, hence there was little difference in PAE. Thus, in this study the P-efficient faba bean variety such as *Moti* produced reasonably high yield in low P soils through PUE and thus can reduce mineral P fertilizer input requirement in agricultural production.

### 6.1.3. Crop responses to P-deficiency

#### *6.1.3.1. Physiological responses*

P deficiency is a primary limitation to legume production in developing countries. Legume genotypes vary substantially in their adaptation to suboptimal P soils, apparently because of differing ability to acquire P from the soil. Different crop species have specific responses of photosynthesis to P deficiency (Thuynsma, 2013). In some plants, the rate of photosynthesis may decline while others can maintain normal rate of photosynthesis (Raghothama, 1999). However, under long term P deficiency photosynthetic rates could decline and plant growth may be impaired to compensate for the P deficiency (Rao and Terry, 1995). Some plants can switch to conserve and recycle phosphate so as to maintain functional levels of photosynthesis (Thuynsma, 2013).

The other most notable effect of P deficiency is on the plant’s physiological activities such as leaf growth and development. Sub-optimal P conditions for common beans (Lynch et al, 1991) and soybeans (Chiera et al., 2002) were related to reduction in leaf growth in terms of both leaf number and individual leaf size. Phosphorus plays an important role in cell division activities. However, plants may also differ in response of P stress effect on leaf growth mainly due to differences in the ability to maintain cell division, leaf epidermal cell expansion or both under lower tissue P concentration (Chiera et al, 2002; Kavanova et al., 2006). Plants may also re-translocate limited P from older leaves to younger leaves to maintain P at levels that allow optimal physiological activities including cell division (Akhtar et al., 2008). In view of these outlooks, the superior grain yield performance of variety Moti may further suggest that Moti may have mechanisms that efficiently translocate P in the internal plant system and avoid too much P storage in the tissue (had lower tissue P concentrations) such that photosynthesis and growth are not limited by P shortage.

#### *6.1.3.2. Root morphological responses*

Phosphorus uptake efficient cultivars may contribute to sustainable crop production by producing reasonably high yield under P deficient condition due to their ability to exploit greater soil volume for accessing more P through producing larger root system (higher root-shoot ratio), longer root hairs or via forming association with mycorrhiza (Balemi and Negisho, 2012). A number of studies on legumes (e.g., soybean, common bean, faba beans) have shown that mechanisms of enhanced P acquisition included enhanced root growth and activity (Lynch, 1995) to thoroughly explore the soil. On low plant available P soils, there may also be a positive interaction between mycorrhizal activity and legumes. Since P is a key nutrient in legume nutrition, populations of mycorrhizae can infect faba bean roots and play an important role in supporting plant growth by assisting the supply of additional P (Jensen et al., 2010).

Studies on common bean genotypes in diverse soils indicated that substantial genetic variation in the growth and architecture of the root systems was evident, and was shown that P-efficient genotypes have a vigorous and highly branched root system with a large number of apices (Lynch, 1995). These varied mechanisms for increased P uptake by plants have led to genetic variation among species for P uptake and P use efficiency. P-efficient genotypes of common bean have shown more shallow roots in the topsoil where there are relatively high contents of P resources (Shen et al., 2011). Some crop species, like white lupin, can develop cluster roots with dense and determinative lateral roots, which are covered by large numbers of root hairs (Vance et al., 2003). Therefore, root architecture plays an important role in maximizing P acquisition because root systems with higher surface area are able to explore a given volume of soil more effectively (Lynch, 1995). These observations may also justify the fact that faba beans in the present study might have altered the architecture of their root systems under P stress conditions to optimize P acquisition and give out high yield.

### 6.1.4. $N_2$ fixation and N balance

Biological $N_2$ fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity (van Kessel and Hartley, 2000). Efficient management of legumes in order to maximize benefits depends on a correct field assessment of $N_2$ fixation. The amount of $N_2$ fixed by the symbiotic relationship between faba bean and the rhizobia is determined by the relative reliance of the crop upon $N_2$ fixation for growth (i.e. %Ndfa) and the amount of N accumulated by the crop over the growing season (Jensen et al., 2010). There is sufficient capacity for BNF to supply the majority of the faba bean N requirements for growth and our field data indicated that $N_2$ fixation can support the accumulation of 8 - 11 t total dry matter $ha^{-1}$.

Total BNF by faba bean varieties also increased with P application and was most pronounced for variety *Moti*, which fixed about 338 kg N $ha^{-1}$. The increased amount of $N_2$ fixed with P application might be due to the increased levels of biomass yield because %Ndfa has slightly decreased with P application. Peoples et al. (2009) also reported that the amount of N fixed is generally controlled by faba bean growth rather than by %Ndfa implying that BNF in faba beans is largely controlled by variety. Further, the contribution of the faba bean to soil N reserves, as measured via the N balance, was positive for all varieties. However, this balance did not take into account the N that is rhizodeposited and hence the estimated soil N balance might have been underestimated. Fixed N contained in attached and detached roots and nodules, and rhizodeposition was essentially ignored (Herridge et al., 2008). It has become increasingly evident that measurement of root N of field-grown legumes is problematic, since it is virtually impossible to recover complete root systems from the field grown plants. Even if it were possible to harvest complete root systems including all fine roots, this would still not capture the dynamic nature of root turnover and exudation of N (Unkovich et al., 2000). In other reports, a factor was used to account for below-ground N (BGN), usually based on a published or experimentally-determined value derived from the physical recovery of roots (e.g. Evans et al. 2001). There is no single value for BGN, with the variation in published estimates reflecting effects of species, soil and climate on the partitioning of N within the plant. To account for BGN when calculating $N_2$ fixation, Herridge et al., (2008) used a multiplication factor of 2.0 for chickpea (assumes 50% of plant N is below-ground), 1.5 for soybean (assumes 33%

BGN) and 1.4 for the remainder of the pulse legumes including faba bean (assumes 30% BGN). Although these factors are approximations, Herridge et al. (2008) argued that the errors associated with their use are far less than the errors associated with ignoring BGN or using values for physically recovered roots. In view of this background, the positive soil N balace that we reported for faba beans ranging from 72.5 kg N ha$^{-1}$ (*CS-20DK*) to 104.3 kg N ha$^{-1}$ (*Moti*) (Chapter 3) may in reality be higher, according to Heridge et al. (2008), by a facor of 1.4.

In general, it was interesting to note that variety *Moti* combined highest PUE and BNF, and gave largest biomass and grain yield. Hence, *Moti* is considered as an optimal 'dual purpose' legume. Most legume breeding programs in Africa look for genotypes that give high yield under low soil P situations. *Moti* meets this requirement and could be an ideal variety for low P soils in the cool humid highland agro-ecosystems where little or no fertilizer is used and where crop production is based on subsistence farming. Here, we emphasize the need to take P requirements of faba beans into consideration when selecting and introducing new varieties for highland agro-ecosystems; hence careful exploration of genetic resources is an essential component for sustainable intensification of cropping systems of smallholder agriculture. Furthermore, when designing more sustainable smallholders cropping systems, farmers' preferences and criteria for selecting different faba bean varieties for use in their cropping systems should also be assessed besides N and P benefits.

### 6.1.5. The faba bean - wheat rotation

As is the case for other legumes, faba bean can deliver an important benefit to cropping systems via its ability to biologically fix atmospheric $N_2$ and its ability to mobilize sparingly available soil P. Provided the soil contains sufficient populations of effective rhizobia, faba bean can accumulate N both from soil and the atmosphere.

In low-input agro-ecosystems, crop residues are often removed from the field to be used as animal feed or as a source of energy, which is also practiced by smallholder farmers in the tropical highlands of Ethiopia. Under such conditions, the net nutrient contribution of faba beans to the soil may even be negative. In order to change these practices, we evaluated two different residue management scenarios to analyze the role of faba beans in a wheat based rotation system. Scenario-I assumed that all the aboveground biomass of faba bean is exported from the fields and scenario-II assumed that all the above ground biomass except grains and empty pods is returned to the soil. The N and P benefit of faba beans to rotational wheat was subsequently assessed. Our results have shown that scenario-I gave a negative net N balance (kg N $ha^{-1}$) in the range of -86.5 ± 5.8 (variety *Degaga*) to -9.4 ± 8.7 (variety *Moti*) with significant differences between varieties. Scenario-II showed that all balances were significantly improved and the varieties were found to positively contribute N to the system in the range 50.6 ± 13.4 (variety *Degaga* ) to 168.3 ± 13.7 (variety *Moti*) kg N $ha^{-1}$, which was equivalent to 110 – 365 kg N $ha^{-1}$ in the form of urea (46% N). Simple N budgets of the faba bean varieties demonstrated large differences regarding potential N returns to soil. Thus, management of faba bean residues is critical in determining the net contribution to soil N balance.

Wheat was grown after faba beans to test whether residue management of high $N_2$ fixing and P efficient faba bean varieties is beneficial to subsequent wheat productivity. Wheat grain yield, grain N and P uptake after various faba bean varieties increased compared to wheat after wheat. The incorporated legume root, nodule and straw might have played a role in improving wheat yield through N and P carryover via mineralization of residue derived organic P and N (Horst et al., 2001; Nuruzzaman et al., 2005). However, the duration between faba bean harvest and sowing the next wheat, the turnover rate of above and below-ground legume N in soil, the timing of the requirement for N by the subsequent crop in relation to the supply of plant-available forms of N, and the prevailing climatic conditions are all factors that will influence the efficiency with which N derived from legume residues will either be utilized for the growth of a following crop, or be lost from the plant-soil system (Crews and Peoples, 2005; Jensen et al., 2010). Since faba beans in the southwest Ethiopia are harvested in November, just after the rainy season, it can be assumed that soil moisture content and temperature are not limiting mineralization of the organic material in the soil. But it can be assumed that little or no N will be lost leaching since residues are incorporated in the dry seaons. Losses via denitrification remain much more speculative. Few studies (e.g., Jensen et al., 2010; Peoples et al., 2009a) have directly followed the fate of faba bean N using $^{15}$N-labeled residues and reported that a subsequent wheat, barley or cotton crop may recover between 11-17% of the plant N remaining after faba bean. However, more research is needed to assess the availability of N from the faba bean residues in Dedo to the subsequent cereals.

Moreover, the faba beans might have positive effects of N sparing as shown by the lower soil N uptake of faba beans compared to wheat in the present study. Very little research work is available on faba bean-wheat rotation for *Nitisols* of southwest Ethiopia. However our results compared well with those obtained by Habtegebrial and Singh (2006) and Habtemichial et al. (2007) in Northern Ethiopia. It is, therefore, possible to argue that the N and P benefits of faba beans to rotational wheat are likely due to mineralization of crop residue derived organic N and P.

Further, it was noticed that between 12 and 16 kg P ha$^{-1}$ could potentially remain in the soil as plant residues after removal of grains and pods while between 6 and 12 kg P ha$^{-1}$ are taken up by the second wheat which suggests a very high coefficient of utilization of P (50 – 68%). The high coefficient of utilizing P may suggest that besides P derived from the soil and plant residue there might be a strong residual effect of the P added as ORGA fertilizer in the legume crop phase. This agrees with previous residual effects of ORGA on teff (Mirutse et al., 2009). In a pot experiment to determine the contribution of P by faba bean residue to subsequent corn using $^{33}$P labeled legume residues, Nachimuthu et al. (2009) have shown that faba bean residues alone contributed about 10% of the total P uptake by corn as compared to 54% by inorganic P fertilizers. These results may suggest that incorporation of the legume residues may not always lead to net P release to subsequent crops.

### 6.1.6. The mesocosm study

In Chapter 3, we demonstrated that in P-deficient farmer’s fields there was considerable varietal difference in grain and biomass yield of faba beans as well as in BNF and PUE. Further in Chapter 4, the potential benefits of cultivation of faba beans to subsequently grown wheat have been also demonstrated. However, there was lack of information concerning interacting effects of climate, edaphic or variety conditions that determined the agronomic performance, BNF and PUE of faba beans in the study area.

Hence, to assess the effect of climate and soil conditions, we performed a mesocosm experiment in which faba bean varieties were grown at two locations with a different microclimate. To assess the soil effect at each of the two locations, we exchanged soil between the two locations. We found strong evidence that grain and biomass yield and nodulation characteristics of faba beans were determined more by the effect of microclimate than soil or variety. The %Ndfa and BNF of faba beans was, however, determined by microclimate, soil type and variety. It was moreover evident that soil type was more important in determining the rhizosphere phosphatases activity than microclimate or variety effects. Our data did not support the suggestion of Johnson et al. (1996) that exuded phosphatase enzymes into the rhizosphere increases P availability and thus increase plant P uptake and PUE. In our study, PAE was determined by microclimate-soil type interaction rather than soil type alone. The latter suggests that mechanism other than rhizosphere phosphatases activity might have been involved in the PAE of faba beans.

It was further shown that both symbiotic and non-symbiotic endophytic bacterial strains were found in association with root nodules of faba beans grown in two soil types and locations. Five different genera were distinguished based on 16S rRNA gene sequencing: *Rhizobium*, *Bacillus*, *Arthrobacter*, *Staphyllococcus* and *Leifsonia*. The host plant - endophytic bacteria associations appeared not to be affected by any of the factors that we considered in the present study. To the best of our knowledge, very little published data (e.g., Kan et al., 2007) is available regarding endophytic bacteria associated with faba beans except some characterization and genetic diversity studies of *Rhizobium leguminosarum* (e.g., Mutch et al., 2003; Mutch and Young, 2004; Tian et al., 2007; Tian et al., 2008). Thus, we recommend a further detailed isolation, characterization and investigation on the role of these endophytic bacteria on the growth, $N_2$ fixation and P nutrition of faba beans.

## 6.2. Future research perspectives

While this thesis research substantially improves our understanding of the role of faba beans in the wheat based cropping system of the humid tropics of southwestern Ethiopia, our findings also indicated several unresolved issues. The main issue is certainly the lack of direct proof of the mechanisms involved in P acquisition and utilization efficiency by faba beans. In most of our studies, we focused on quantitative analysis of the variation that exits in the faba bean varieties with respect to BNF, PAE and PUE, yet we did not study the possible mechanisms involved in PAE and PUE by faba beans. Although we analyzed the rhizosphere phosphatase activities at two different soils, it was not related to an improved PAE indicating that other potential mechanisms are involved in PAE (e.g., P solubilizing rhizobacteria or mycorhizal association). Therefore, in order to better exploit the potential of faba beans in cropping systems, it will be imperative to further address the following unresolved issues: (1) How do faba beans respond to P deficiency in relation to plant and root growth traits; (2) The rate of N fixed that is rhizodeposited by faba beans; (3) Since farmers in the tropical highlands usually remove the biomass (crop residue) from the field during harvesting to use as animal feed or as a source of energy, it is moreover necessary to analyse the tradeoffs between leaving crop residues in the field and taken them away as farmers presently practice.

Further, we have shown that incorporation of crop residue has positive effects on subsequent wheat. However, the use efficiency of organic N and P might not be as high as that of fertilizer N and P. So, the N balances and P content of the legume residue of the precursor faba bean varieties might not be enough to reach maximum potential cereal yields as with mineral fertilizers. Combining organic and mineral inputs has been advocated as a sound management principle for smallholder farming in the tropics since neither of the two inputs is usually available in sufficient quantities and both inputs are needed in the long-term to sustain soil fertility and crop production (Vanlauwe et al., 2012). Chivenge et al. (2011) reported that maize yield responses and N use efficiency were greater following the combined application of organic residues with low quantities N fertilizers compared to the addition of either resource alone, offering potential for increasing crop productivity. In the context of ISFM, it is therefore important to see the possible benefits from a combined application of crop residue and a smaller quantity of fertilizers N and P given to the cereal grown after the faba bean.

In the present study, it was found that microclimate had a profound effect on the performance of faba beans such that average grain yield, for instance at Dimtu (lower altitude) was four times lower than that obtained at Dedo (higher altitude). This large yield difference between the locations was due to differences in temperature variations of the respective locations. Rainfall distribution was more or less similar. According to Patrick and Stoddard (2010), temperature has an effect on flowering, flower retention and seed formation which are key processes determining yield. This might necessitate the search for faba bean varieties better adapted to high temperatures so that faba beans continue to have positive ecological services in the cropping system. Moreover, management practices such as adjusting the time of sowing can influence the time of flowering and pod fill, thereby minimizing the effect of terminal drought. Alternatively, screening and introduction of other tropical grain legumes (e.g., common beans, soybeans) for the lower altitude might warrant sustainability of smallholder cropping systems.

BNF of faba beans was assessed in a sole cropping system. It is well documented that intercropping may increase the efficiency of BNF of legume compared with its monoculture (e.g., Fan et al., 2006). One of the prime objectives of contemporary intercropping studies is to assess the N economy of the component crops in intercropping. It is therefore essential to see how BNF of faba beans responds to nutrient facilitation in faba bean/cereal (e.g., wheat, teff) intercropping systems.

Finally, using the present study as baseline, further research on the role of faba beans in other important cereals in the region (e.g., teff, barley) could lead to a broader positive impact of faba beans in the highland agro-ecosystem.

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# CURRICULUM VITAE

## Amsalu Nebiyu WoldeKirstos

1. **Personal**

   **Date of birth**: Born 31 December 1971

   Place of Birth: Amanuel-Gojjam, Ethiopia

   Gender: Male

   Nationality: Ethiopian

   Marital status: Married

2. **Address:**

   College of Agriculture and veterinary medicine, Jimma University
   PO Box 1316, Jimma, Ethiopia
   Tel: 00251-47-1118332 (home); Cell: 00251-91-7804332
   Fax: 00251-47-1110934
   e-mail: anebiy@yahoo.com; amsalu.nebiyu@ju.edu.et

3. **Education**:

| **Institution** | **Degree** | **Year** | **Specialization** |
|---|---|---|---|
| University of Ghent, Belgium | *PhD; on study | 2009- to date | Applied Biology (Agronomy/Soil fertility) |
| Alemaya University, Ethiopia | MSc | 2003 | Agriculture (Horticulture) |
| Alemaya University, Ethiopia | BSc | 1998 | Agriculture (Plant sciences) |

**To be completed by Feb 2014*

4. **Employment/work experience**:

- September 1998 – April 1999: Coffee Agronomist and Instructor of plant sciences at the then Coffee and Tea authority of Ethiopia.
- May 1999 - September 2004: Researcher (In Horticulture and plant sciences) at the Ethiopian Institute of Agricultural Research, Jimma Research Center.
- October 2004- January 2009: Lecturer and researcher at the College of Agriculture and

veterinary medicine, Jimma University

- February 2009 to date: PhD fellow, university of Ghent, Belgium

## 5. Professional Societies:

- Active member of the Crop Science Society of Ethiopia (Since 1999)
- Active member of the Ethiopian Horticultural Sciences Society (Since 2006)
- Active member of the Ethiopian Soil Science Society (Since 2008)
- Student member of the Soil Science Society of Belgium (Since February 2009)
- Member of The Cassava Cyanide Diseases Network (Since 2003)

## 6. Leadership role

- Project leader: Soil fertility project funded by Inter University Cooperation Programme, (VLIR-IUC) (Belgium)-Jimma University (JU) (VLIR-IUC), May 2009 – to date)
- Deputy project leader: Soil fertility project (VLIR-IUC): (January 2006-April 2009)
- Head research and Publications, College of Agriculture, Jimma University (April 2005 – Sept 2008)
- National Project leader: Indigenous Root and Tuber Crops Research project (Ethiopia) (2002-2004)

## 7. Publications:

### *7.1. Peer-reviewed Journal papers*

1. Amsalu Nebiyu, Dries Huygens, Hari Ram Upadhayay, Jan Diels and Pascal Boeckx (2013). Importance of variety specific B-values to quantify biological N2 fixation of faba beans (*Vicia faba* L.) via $^{15}$N natural abundance. *Biology and fertility of soils-* DOI 10.1007/s00374-013-0874-7 (in press).
2. Amsalu Nebiyu, Adeline Vandorpe, Jan Diels and Pascal Boeckx (2013). Nitrogen and phosphorus benefits from faba bean (*Vicia faba* L.) residues to subsequent wheat crop in the humid highlands of Ethiopia. *Nutrient cycling in agroecosystems* (under revision).
3. Amsalu Nebiyu, Dries Huygens, Davy Polfliet, Anne Willems, Jan Diels and Pascal Boeckx. Climatic, edaphic or variety controls on biological N2 fixation and P use efficiency faba beans (*Vicia faba* L.). *Agriculture, ecosystem and environment* (under review)

4. Amsalu N. and Essubalew G. 2011. Soaking and drying of cassava roots reduced cyanogenic potential of three cassava varieties at Jimma, Southwest Ethiopia. African Journal of Biotechnology 10(62):13465-13469.
5. Sirawdink F., Zerihun K., Amsalu N., Nardos Z., and Seife B. 2011. Allelopathic Effects of *Eucalyptus camaldulensis* Dehnh on Germination and Growth of Tomato. American-Eurasian J. Agric. & Environ. Sci., 11 (5): 600-608
6. Amsalu Nebiyu. 2006. Genetic Variations in Cassava at Jimma, Southwest of Ethiopia. Tropical Science 46(3):171–175.

### *7.2. Conference attendance and paper presentation during the PhD study period*

1. Amsalu Nebiyu, Upadhayay, H.R., Diels, J., Boeckx, P., 2012. B-value and isotopic fractionation during N2-fixation by faba beans. In: Woomer, P.L. (ed.). Integrated Soil Fertility Management in Africa: from Microbes to Markets: Conference Information, Program and Abstracts. An international conference held in Nairobi, Kenya, 22-26 October 2012. CIAT. 122 pp.

2. Amsalu Nebiyu, Vandorpe, A., Diels, J., Boeckx, P., 2012. Response of wheat to N2 fixation by preceding faba bean and applied P grown in rotation in the tropical highlands of southwest Ethiopia. In: Woomer, P.L. (ed.). Integrated Soil Fertility Management in Africa: from Microbes to Markets: Conference Information, Program and Abstracts. An international conference held in Nairobi, Kenya, 22-26 October 2012. CIAT. 122 pp.

3. Amsalu Nebiyu, Hari Ram, Adeline Vandorpe, Jan Diels and Pascal Boeckx. 2011. Comparative Performance of elite Faba Bean (Vicia faba L.) Varieties to Grain Yield and Biological Nitrogen Fixation in the Humid Highland Systems of Southwest Ethiopia. A Poster presented at the International Conference on 'Challenges and Opportunities for Agricultural Intensification of the Humid Highland Systems of Sub-Saharan Africa, Kigali, Rwanda, 24th – 27th October 2011.

4. Amsalu Nebiyu, Hari Ram, Jan Diels and Pascal Boeckx. 2011. Soil P fractions and P use efficiency of faba beans (Vicia faba L.) in the highlands of southwest Ethiopia. A Poster presented at the International Conference on 'Challenges and Opportunities for Agricultural Intensification of the Humid Highland Systems of Sub-Saharan Africa, Kigali, Rwanda, 24th – 27th October 2011.

5. Amsalu Nebiyu, Jan Diels and Pascal Boeckx. 2010. Performance of elite Faba Bean (Vicia faba L.) varieties at two different altitudes on Nitisols of south western Ethiopia. Paper presented at the annual conference of the young soil scientists day organized by the Soil Science Society of Belgium, 23 Feb 2010, Brussels, Belgium

## 8. Conference attendance and paper presentation before the PhD study period

6. Amsalu Nebiyu and Tesfaye Awas. 2006. Explorations and Collection of Root and Tuber Crops in South-western Ethiopia: Its Implication for Conservation and Research. Pp 84-88. In: Proceedings of the 11th Conference of the Crop Science Society of Ethiopia, 26-28 April 2004, Addis Ababa, Ethiopia

7. Amsalu Nebiyu. 2006. Phenotypic diversity of cassava in Ethiopia. Paper presented at the 12th Conference of the Crop Science Society of Ethiopia, 23-24 May, 2006, Addis Ababa, Ethiopia

8. Amsalu Nebiyu and Elfinesh Frdisa. 2006. Effects of different proportions of maize and Soybean flours on cassava bread quality characteristics. Paper presented at the 3rdh National Horticulture Workshop, 27-30 March, 2006, Addis Ababa, Ethiopia

9. Amsalu Nebiyu. 2004. Genetic Diversity of Cassava in Ethiopia: Its Implication for Food Security and the Need for Biotechnology Research, Pp. 39, In: The six International Scientific Meeting of the Cassava Biotechnology Network, 8-14 March, 2004, CIAT, Cali, Colombia (Abstarct).

10. Amsalu Nebiyu. 2003. Characterization and Divergence analysis in Cassava (Manihot esculenta Crantz) Accessions at Jimma. MSc Thesis, Alemaya University, Ethiopia.

11. Amsalu N. and Edossa E.2002. Achievements in Root and tuber crops improvement Research and Future Prospects in South-western Ethiopia. Pp 158. In: Proceedings of the horticulture seminar on sustainable horticultural production in the tropics. 3rd-6th

Oct 2001. Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology.

12. Amsalu N. and Edossa E. 2002. Agronomic studies for sustainable pineapple production in South-western Ethiopia. Pp 159. In: Proceedings of the horticulture seminar on sustainable horticultural production in the tropics. 3rd-6th Oct 2001. Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology.

13. Wondifraw T., Dawit A., Haileab A., Amsalu Nebiyu and Tirfalem H.W. 2006. Effects of Stand Regulation on yield and quality of pineapple. Paper presented at the 3rdh National Horticulture Workshop, 27- 30 March, 2006, Addis Ababa, Ethiopia