Improving Snap Bean (*Phaseolus vulgaris* L.) Production under Reduced Input Systems

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ABSTRACT

Snap bean (*Phaseolus vulgaris* L.) production by large scale commercial producers in Ethiopia is under intensive production and relies on high rates of nitrogen (N) fertilizer and irrigation during the dry season. Despite increasing interest to produce this crop, small scale farmers cannot afford the high cost of N fertilizer. Field and greenhouse experiments were conducted to test snap bean production under a low input production system better suited to small scale resource limited farmers.

Field experiments were conducted in 2011 and 2012 under rain fed conditions, and in 2012 under irrigation, at three locations (Debre Zeit, Hawassa, Ziway) representing different climate zones in Ethiopia. This experiment used three N treatments: 0 and 100 kg N ha^{-1}, and inoculation with *Rhizobium etli* [HB 429], and eight cultivars: Andante, Boston Contender Blue, Lomami, Melkassa 1, Melkassa 3, Paulista and Volta. The general objective of the field experiment was to determine the potential of snap bean production under a low input production system using rhizobium inoculation as the nitrogen source, and use rain fed conditions. Results obtained indicated that rhizobial inoculation and applied inorganic N increased on average the marketable pod yield of snap bean under rain fed conditions by 18 % and 43%, respectively. Nodulation and subsequent N_2 fixation was not effective in improving yield or other traits of snap bean pod under irrigation, although applied N increased marketable yield by 33%. Melkassa 1 was the most suitable cultivar for a reduced input production system due to its successful nodulation characteristics, greatest N_2 fixation levels and consistently good performance across locations under rain fed conditions. Commercial cultivars possessed the best pod quality characteristics and they yielded better under irrigation. Cultivars interacted with locations to affect pod traits including total soluble solids and concentrations of protein, calcium, and potassium under rain fed conditions. Snap bean cultivrs produced at Debre Zeit and Hawassa were similar in marketable yield and several other traits particularly under rain fed conditions. Zinc (Zn) concentration in pods was greatest at Hawassa both under rain fed and irrigated conditions. Conditions at Debre Zeit were the most conducive for supporting biological N_2 fixation for snap bean production.

The eight cultivars were also used for a greenhouse study that was evaluated treatments of drought stress of 50% field capacity (50% FC) during the vegetative (V4.4), flowering (R6) and pod formation (R7) developmental stages. Our result showed that drought stresses during

reproductive stages (R6 and R7) were the most sensitive stages in deteriorating the quality of snap bean pods. Drought stress increased protein, phosphorus and Zn concentrations but it reduced iron concentration in snap bean pods. All cultivars had a similar response to drought stress. A second greenhouse experiment was conducted to test foliar application of growth regulators: the control, 10^{-5} M and 10^{-4} M concentrations of each of abscisic acid (ABA), kinetin and salicylic acid (SA); and two concentrations of yeast extract (4 g l^{-1} and 8 g l^{-1}), under drought (50% FC) stressed and unstressed conditions. Foliar application of SA on snap bean under greenhouse conditions reduced the impact of drought stress, particularly the pod quality parameters: marketable yield, pod curving, texture and appearance of snap bean pods. However, application of ABA, kinetin and SA reduced pod quality of snap bean under unstressed conditions.

In conclusion, pod yield improvement could be achieved by a N_2 fixation system under rain fed conditions, which is more sustainable than N fertilizer inputs. Pod quality was also adequate for commercial export production. Rhizobium inoculant can therefore be used as an alternative N source, particularly under low input production system for resource-limited small-scale snap bean producers.

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%Ndfa :	Percentage of Nitrogen Derived from the Atmosphere
^{15}N (‰) or $\delta^{15}N$:	Proportion of concentration (percentage) of ^{15}N in a sample compared to its concentration in atmosphere
ABA:	Abscisic Acid
AIC:	Akaike Information Criterion
ANOVA:	Analysis of Variances
Ca:	Calcium
CK:	Cytokinin
CIAT:	International Centre for Tropical Agriculture
CRD:	Completely Randomized Design
DDFM:	Degrees of Freedom Method
et al.:	et alia (and others)
FAOSTAT:	Food and Agriculture Organization, United Nations statistical Database
FC:	Field Capacity
Fe:	Iron
g:	Gram
GA_3:	Gibberellic acid
h:	Hour
ha:	Hectare
IAA:	Indole-3-acetic acid
K:	Potassium
kg:	kilogram
l:	Liter
LSD:	Least Significant Difference
m:	Meter
M:	Molar
MARC:	Melkassa Agricultural Research Centre
mm:	Millimetre
MDA:	Malondialdehyde

Mt:	Million Tonnes
N:	Nitrogen
NaOH:	Sodium Hydroxide
ns:	Not Significant
P:	Phosphorus
ppm:	Parts per million
RCBD:	Randomized Complete Block Design
SA:	Salicylic Acid
SAS:	Statistical Analysis System
t:	Tonne
TA:	Titratable Acidity
TSS:	Total soluble Solids
USDA	United State Department of Agriculture
V:	Cultivar
Zn:	Zinc

CHAPTER 1

1. Introduction

Common bean (*Phaseolus vulgaris* L.*)*, is a herbaceous annual plant domesticated independently in ancient Mesoamerica and in the Andes, and now is grown worldwide for both dry seeds or as a green bean. Thousands of legume species exist but common bean in any form is the most eaten by human beings compared to any other legume (Broughton et al., 2003).When common bean is used for its unripe fruit, it is termed as green bean or snap bean. About 23.9 Mt of dry bean, 20.7 Mt of green (faba) bean, and 1.9 Mt of string or snap bean were produced worldwide in 2012 (FAOSTAT, 2014). Snap bean is characterized by succulent and flavorful pods (Stephen, 1998). They are one of the most important legume vegetable crops and contributes substantial amount of protein to human diet. Nitrogen fixation and the subsequent internal supply of nitrogen (N) from their symbiosis with rhizobia make legume crops richer in protein in dry weight basis than all other plants (Broughton et al., 2003).

In Ethiopia, snap bean is economically one of the most important vegetable crops grown for both export and local markets. It is mostly grown in the Rift Valley region, especially for export. In 2012, Ethiopia exported an estimated production quantity of about 6200 t of snap bean mainly to Europe and the Middle East, bringing a considerable amount of revenue to the country (FAOSTAT, 2014). Snap bean production in Ethiopia has increased from time to time both for export and local markets. In addition to large commercial vegetable farms which produce snap bean for export, snap bean is increasingly popular for small-scale vegetable producers for local markets. Snap bean producers for the export market are restricted in their production by having to use irrigation during the dry season (October to April). The potential of snap bean production during the main rainy season has not been evaluated under Ethiopian conditions. This information is needed to expand the production of snap bean into different seasons and the many climate zones/agro-ecologies of Ethiopia.

Currently, snap bean production in Ethiopia relies on a few introduced cultivars from Europe and America. The agronomic practices of the introduced cultivars are usually based on the package developed by the seed companies in the respective countries. These agronomic practices are usually difficult to apply in the Ethiopian context, especially by small-scale farmers due to limited resources. Commercial snap bean production depends heavily on applied N

fertilizer. Relatively high rates of N fertilizer are applied regardless of the cultivars and other factors such as residual soil N. The majority of Ethiopian farmers, however, are unable to afford the high mineral fertilizer cost. Biological N_2 fixation, a key source of N for poor farmers, constitutes a potential solution and may play a key role in sustainable bean production in sub-Saharan Africa (Chianu et al., 2011).

The benefits of using rhizobia for N_2 fixation have been realized in chickpea (*Cicer arietinum* L.) (Bhuiyan et al., 2008), soybean (*Glycine max* Merr.; Sall and Sinclair, 1991), common bean *(P. vulgaris* L.) (Bildirici and Yilmaz, 2005; Otieno et al., 2009), and many other legumes. Reports have shown that effective N_2 fixation by rhizobia and further conversion into yield is affected by the genotype of the host plant in common bean (Bildirici and Yilmaz, 2005; Sadowsky et al., 1988), and environmental factors such as drought in soybean (Purcell et al., 1997). Previous studies were focused on the use of N_2 fixation to improve grain yield of legume crops. Reports are lacking on the use of rhizobial inoculant as source of N for vegetable legumes particularly snap bean. Snap bean is harvested at horticultural maturity long before the end of season unlike grain crops. Whether N_2 fixation sustained until horticultural maturity is sufficient to benefit yield and quality improvement of snap bean should be investigated. Therefore, the goal of the current study was to evaluate the potential of using rhizobium inoculation to provide sufficient N in low input snap bean production systems.

Drought stress can happen at any time and at any stage of snap bean growth to adversely affect yield and quality of the produce, especially under rain fed conditions (Katungi et al., 2010). Occurrence of drought stress during the growing period is one of the most devastating factors in dryland crop production. The most common types of drought stress in eastern Africa are midseason and terminal droughts (Katungi et al., 2010). The extent of damage to a crop due to drought stress depends on several factors such as growth stage, type and magnitude of the drought stress, duration and timing of the stress. Some cultivars of common bean tolerate moisture stress better than others (Molina et al., 2001). Currently, there is no information on the relative response of current snap bean cultivars to moisture stress, and whether the response to the stress would be similar across different growth stages. It is important to know the levels of drought stress that can be tolerated by different cultivars of snap bean. Identifying the most sensitive growth stage of snap bean plant to drought stress is critical to reduce risk. This information is needed to determine the best timing for irrigation which will eventually increase

water use efficiency, save labour and save capital, which are all critical constraints in current snap bean production in Ethiopia. The appropriate time of planting snap bean to avoid drought stress during the most sensitive stages of development under dryland production can also be deduced.

Dryland snap bean production could contribute to economical and nutritional benefits particularly for small-scale resource-limited farmers. However, drought stress is a major problem in bean growing areas of eastern Africa (Katungi et al., 2010), and technology and management practices should be designed to minimize the impact of stress. Several studies have demonstrated that application of exogenous growth regulators including salicylic acid (SA) (El-Tayeb and Ahmed, 2010; Habibi, 2012; Hayat et al., 2012; Kabiri et al., 2012; Sadeghipour and Aghaei, 2012), and cytokinin (CK) (Thomas et al., 1992; Pospisilova et al., 2000; Hare et al., 1997; Metwally et al., 1997) reduces the impact of drought stress. Salicylic acid, CK and abscisic acid (ABA) play an important role in drought tolerance (Farooq et al., 2009b). Application of yeast extract has additionally been reported as having a positive effect on improving yield and quality in different crops (El-Yazied and Mady, 2012; Shehata et al., 2012; Abbas, 2013). However, the response of snap bean to these growth regulators is not yet known under transient or moderate drought conditions prevalent in production for export quality.

1.1 Hypotheses

1. Rhizobium inoculation can be the main N source to produce high quality snap bean under low input production systems and cultivars vary in nodulation and N_2 fixation.
2. Drought stress during different developmental stages of snap bean can affect pod yield, quality and nutrient concentrations and cultivars vary in relative tolerance for drought.
3. Foliar application of growth regulators can reduce the impact of drought stress on pod yield, quality and nutrient concentrations of snap bean.

1.2 General objectives

1. To determine the potential of snap bean production under low input production systems using rhizobium inoculation as the main N source under rain fed conditions.
2. To determine the impact of drought stress on snap bean yield and quality performance.
3. To test the response of snap bean to growth regulators under transient drought conditions during reproductive growth.

1.3 Specific objectives

1. To test the effect of use of rhizobia inoculation as an alternative N source for snap bean production under rain fed conditions.
2. To assess the possible use of rhizobium inoculation as the main source of N to produce quality snap bean pods relative to the commonly used dose of synthetic N fertilizer and the control treatment without N.
3. To identify suitable cultivars across contrasting environments for reduced input snap bean production those that have specifically N_2 fixation.
4. To evaluate cultivars for marketable yield, pod physical qualities and nutrient concentrations under rain fed conditions across contrasting environments.
5. To investigate the influence of N treatments and cultivars on pod quality of snap bean under irrigated conditions across contrasting environments.
6. To evaluate the impact of drought stress at different growth stages on physical pod qualities.
7. To investigate the influence of drought stress on nutrient concentrations of zinc, iron, protein, calcium, phosphorus and potassium in snap bean pods.
8. To evaluate the relative tolerance of snap bean cultivars to drought stress at different developmental stages and identify the most sensitive stage of development to drought stress.
9. To determine the effect of foliar applications of the growth regulators ABA, kinetin, SA and yeast extract to reduce the impact of drought stress on snap bean yield and quality.

CHAPTER 2

2. Literature Review

2.1 Crop Characteristics

Common bean (*P. vulgaris* L.) belongs to the legume family (Fabaceae) and is among the oldest and most important food crops in the world, both nutritionally and economically (Cotner, 1985). Snap bean is usually called garden, green, wax or string bean. All are grown for their immature pods. The crop is a herbaceous annual with alternate trifoliate leaves. Morphologically it is grouped into determinate (bush), half (semi-determinate) runner and indeterminate (pole) types based on the growth habit. The determinate types are short erect plants with a height of 25 to 38 cm and spread of 10 to 20 cm (Decoteau, 2000). Their growth is terminated with reproductive meristem (inflorescence). Flowering in cultivars having a determinate growth habit is concentrated (usually 5-6 days). These cultivars are used for short-season production, and successive plantings every two weeks are needed for a continuous supply. Snap bean cultivars for processing are also determinate types for one-time picking by mechanical harvest. Indeterminate types develop vines that must be supported by a fence or stakes, or be grown on a trellis. They continue to grow after entering reproductive stage. Flowering in indeterminate type extends for 15 - 30 days. The half runner types combine the characteristics of both determinate and indeterminate types and referred as semi-determinate types.

Flowers of snap bean are borne axially, the corolla may be white, creamy-yellow, pink or violet. The flowers are usually self-fertile and pollination takes place at the time when the flower opens. Most snap bean cultivars are day neutral; however, many indeterminate types are short day (Kay, 1979). Snap bean pods are normally ready for harvest 50 - 60 days after planting, some 14 - 28 days after the first flower appeared, even though variation occurs in different altitudes and cultivars (Kay, 1979).

2.2 Snap bean production

Common bean (*P. vulgaris* L.) has been evolved over at least a period of 7000 – 8000 years from a wild ancestral form distributed in the highlands of what is now Latin America between northern Mexico and Northern Argentina (Gepts and Debouck, 1991). Before domestication,

wild *P. vulgaris* had already diverged into two major gene pools, each with characteristic geographic distribution, in Mesoamerica and Andes. In addition to these two major gene pools, recently discovered wild bean populations constitute a third, distinct germplasm segment as intermediate gene pool from Southern Colombia, Ecuador and Northern Peru (Debouck et al., 1993; Gept, 1998). The crop is grown worldwide for its edible bean, and is popular as dry, fresh or green bean. The common bean as grain is high in starch, protein and dietary fiber and is an excellent source of minerals and vitamins including iron (Fe), potassium (K), selenium, molybdenum, thiamine, vitamin B6, and folic acid. Common bean is grown as a grain throughout Ethiopia and is an increasingly important commodity in the cropping systems of smallholder producers for food security and income (Ferris and Kaganzi, 2008). When common bean is used for its unripe pods, it is termed as green bean or snap bean. Snap bean cultivars are characterized by succulent and flavorful pods (Stephen, 1998). Snap bean is one of the important vegetable crops used as a source of foreign currency. More than 90% of snap bean produced in east Africa is exported to within Africa and to other international markets (CIAT, 2006). Snap bean pods are the largest vegetable exported from Africa to Europe.

Snap bean production in Ethiopia both for export and for local consumption was started very recently even if local people had traditionally consumed common bean seeds at the green pod stage. A cereal based food tradition and lack of knowledge about the nutritional value limited this crop as a vegetable crop. However, production of snap bean in Ethiopia for international markets and local consumption has been increasing in the last decade. Commercial snap bean production in Ethiopia is concentrated during the off season of European countries (October to April) due to less market competition from within the European countries. Currently, snap bean production in Ethiopia is gaining importance for international markets along with other African countries such as Kenya, Tanzania and Uganda (CIAT, 2006). The destination of snap bean produced in Ethiopia is not limited to Europe but rather is being expanded to the Middle East and other Arab countries. Hence, snap bean can be exported from Ethiopia at any time of the year provided that high quality pods are produced.

According to Norman et al. (1995) productivity and quality of snap bean were determined by a number of factors such as the choice of adapted cultivar to a particular location, and other agronomic and biological factors. Snap bean production in Ethiopia depends either on foreign

packages or farmers' own experiences, and small individual farmers experience low productivity and pod quality. Furthermore, snap bean production in Ethiopia is constrained by a lack of suitable cultivars in terms of yield, quality and disease resistance for the different agro-ecological zones, a lack of infrastructure and transportation facilities, the absence of local markets and a poor knowledge of the nutritional value of the crop. Research and extension in Ethiopia has traditionally focused on staple cereals, coffee, and livestock sectors, giving limited support to the horticultural sectors, including snap bean production.

2.3 Nitrogen and N_2 fixation

The high dependence of modern agriculture on synthetic fertilizer as a N source has resulted in multiple problems. Dramatically increased price, release of greenhouse gases including carbon dioxide during the combustion of fossil fuels and release of nitrous oxide, losses due to inefficiency of application resulting in environmental pollution are some of the major constraints (Reid et al., 2011). Therefore, there is a strong need to minimize the reliance on chemical N fertilizer and instead optimize alternative N inputs (Ferguson, 2013; Ferguson et al., 2010).

Snap bean is a legume crop, but it requires some N fertilizer to maximize yield (Slaton et al., 2007). Mineral nutrients are essential for growth, development and productivity. Nitrogen is essential for synthesis of chlorophyll, enzymes and protein (Devlin and Witham, 1986). Application of the recommended rate of fertilizer on snap bean plants increased yield and improved pod quality (El-Awadi et al., 2011). Salinas-Ramírez et al. (2011) reported that N fertilizer at a rate of 200 kg ha^{-1} in snap bean increased yield, biomass production, phosphorus (P) and protein. Results also demonstrated that pod quality (pod length and weight) and nutritive value (N, P, K, total soluble solids (TSS), protein and carbohydrate contents) were gradually and significantly increased by increasing the level of N application up to 330 kg N ha^{-1} on snap bean (Mahmoud et al., 2010). Nitrogen from various sources such as rhizobium inoculation, organic and inorganic fertilizers increased protein and tannin content of faba bean (*Vicia faba*) seed; however, these reduced the carbohydrate content (Elsheikh and Elzidany, 1997). In addition, research by Osman et al. (2010) indicated that inoculation with rhizobium resulted in increased faba bean seed yield, ash, fat, crude protein, and 100-seed weight. However, excessive N

fertilizer may increase water pollution (Davidson et al., 2012), diminish methane uptake by microbes, and reduce vitamin C (El-Otmani and Ait-Oubahou, 2011) content of plant products.

Given the high cost of fertilizers in Africa and the limited market infrastructure for farm inputs, current research and extension efforts have been directed towards integrated nutrient management, in which legumes play a crucial role (Chianu et al., 2011). Inoculation with compatible and appropriate rhizobium may be necessary where a low population of native rhizobial strain predominates and is a solution for legume farmers to optimize yield. Rhizobia are the dominant symbiotic N_2 fixing bacteria with legumes but a number of factors, including low numbers of appropriate rhizobia and competition from ineffective native rhizobia, can lead to poor nodulation and N_2 fixation in legumes. Common bean can fix significant amounts of N_2 through biological N_2 fixation provided that the plant is not constrained by high temperature, lack of P supply, presence of stresses and toxic elements (Thung and Rao, 1999). One report by Giller et al. (1998) indicated that rhizobium inoculation of common bean increased nodulation and N_2 fixation but did not increase seed yield. Another investigation showed that nodule number and dry weight were increased by rhizobium inoculation but inoculation had no significant impact on yield (Otieno et al., 2009). Diouf et al. (2008) discussed variability in common bean cultivars for their response to rhizobium inoculation for efficient biological N_2 fixation. Bildirici and Yilmaz (2005) reported that yield and yield components of common bean were positively affected by rhizobia inoculation. However, their work also indicated that number of seeds per pod and thousand seed weight were highly dependent on crop genetics. The change in number of seeds per pod and thousand seed weight due to environmental factors such as rhizobium inoculation and fertilizer application was very small. According to an Organic Seed Alliance (2007) report, some growers in different countries inoculated their bean seeds with rhizobium inoculants prior to planting. This practice can be particularly beneficial when production occurs on soils where bean have not been grown in the past, or in soils that are low in biological activity. According to research from Senegal, the population of effective rhizobium strain was affected by differences in agro-ecologies (Diouf et al., 2008).

Rhizobia symbiosis with common bean is highly determined by host genotype (Sadowsky et al., 1988). This study indicated that the greatest nodule number and weight and accumulated N were observed only in certain genotypes of common bean. Genotypes able to

secrete more flavonoids provided better host to rhizobium leading to better nodulation (Rengel, 2002). Kellman (2008) reported that rhizobium inoculation significantly increased the 1000 seed weight of common bean, especially under greenhouse conditions. Under optimized environmental conditions, genetically superior genotypes of common bean that are nodulated with efficient rhizobium strain are able to fix enough N_2 to support grain yield (Bliss, 1993b). Rhizobium inoculation in chickpea resulted in a significant increase in yield and nodule parameters of the plant (Bhuiyan et al., 2008). Hirsch (1992) indicated that legume plants form nodules only when they are grown in soil that is nitrogen-deficient and then infected by a bacterium that is compatible with the host plant.

2.4 Environment/Agro-ecological variables for snap bean production

The performance and yield of crop plants are related to the environment through a combination of linear and non-linear responses (Campbell and Norman, 1989). In some cases, as much as 80 % of the variability of agricultural production is due to variation in weather, especially under rain fed conditions (Fageria, 1992; Hoogenboom, 2000). Agro-meteorological variables such as rainfall, soil and air temperature, relative humidity, wind and solar radiation have major impacts on plants, pests and diseases (Hoogenboom, 2000). In turn, these influence crop growth and development and are some of the primary determinants of yield (Dapaah, 1997). Individual legume species or cultivars often require specific ecological niches for maximum production (Masaya and White, 1991), which should not be ignored when considering site suitability whether at local, national or international levels (Valentine and Matthew, 1999). In crop production, one of the first requirements is the appropriate agro-ecological zone which is compatible with the moisture, temperature and soil requirements of the species or cultivar to be grown. A sound knowledge of the developmental and environmental factors contributing to yield and quality variation is therefore required to maximize yield of agricultural crops. Yield variation was observed among different common bean genotypes in east Africa (Tanzania) among different locations (Giller et al., 1998). According to Vilela et al. (2011) environment affects plant height, pod length, pod fiber content, pod diameter, pod weight and some other parameters in snap bean.

Bean production areas in Ethiopia can be broadly classified into four agro-ecological zones: the central, eastern, southern and western zones grouped according to altitudes, rainfall,

soil, production systems and geographical locations. Production constraints, both biotic and abiotic are specific, though some local varieties with low potential yield and susceptibility to pest and diseases are common to all zones. Similarly, preferences and types of bean grown vary in different zones (CIAT, 2003).

According to the Ministry of Agriculture (2000) there are seven moisture regimes in Ethiopia. These are arid (100-800mm), semi-arid (300-800), sub-moist (300-1400mm), moist (600-2200mm), sub-humid (700-2200mm), humid (900-2200mm) and pre-humid (1100-2200) and six thermal zones such as hot (>27.5^{o}C), warm (27.5-21^{o}C, tepid (21-16^{o}C), cool (16-11^{o}C), cold (11-7.5^{o}C) and very cold (<7.5^{o}C), which delineate Ethiopia into 18 major and 49 sub-agro-ecological zones.

Snap bean is adapted to a wide range of altitudes between 1200 – 2000 m above sea level (Wortmann et al., 1998). It is a warm season crop that thrives in frost free climates with warm days and bright sunlight (USDA, 1995). Warmer temperatures (19-27^{o}C) are suitable for most cultivars (Rice et al., 1990). Snap bean can also grow well on most types of soil from light sand to heavy clay, and many cultivars tolerate a range of soil conditions with optimum pH from 5.5 – 6.5. In regions where common bean is grown, the annual precipitation ranges from 400-1200 mm per annum and good yield depends on moderate but well distributed rainfall. The water requirement of common bean is high during the pod filling stage (Raemaekers, 2001).

There is tremendous opportunity for the development of horticulture in Ethiopia. The country has a favorable climate, abundant land, labor and water resources that have all created opportunities for horticultural production including snap bean. Apart from traditional intensive horticultural production, vegetable crops could play a major role for small-scale producers who are mainly dependent on dryland agriculture.

2.5 Pod quality of snap bean

Acceptable snap bean quality includes well-formed and straight pods, bright in color with a fresh appearance, free of defects, tender (not tough or stringy) and firm (Cantwell and Suslow, 1998). Pod appearance, texture and curvature are the major physical qualities that directly influence pod quality for the fresh market.The diameter of the pod, rather than length, is a good indicator of quality. Buyers prefer pods with no or only slight bulges that indicate tender, young

seeds. As the name implies, snap bean should break easily when the pod is bent, giving off a distinct audible snap. Market preferences for snap bean pods differ with regions. Most of the snap bean pods produced in eastern Africa are round and thin mainly to suit European markets. Snap bean improvement in eastern Africa focuses on the development and production of bush and climbing snap bean cultivars with a high proportion of the harvestable yield of each plant being extra fine and fine bean pods that command premium prices. However, increasing productivity can sometimes be associated with later maturity period or an increase in pod size and decrease in quality (Myers and Baggett, 1999).

Snap bean is considered one of the important vegetable crops cultivated in many African countries for local markets and it has great importance for export. However, bean plants are relatively sensitive to environmental stresses that may occur in the field compared to most vegetable crops which negatively affect its growth, yield and even the quality of pods.

Snap bean pods are a food source that can contribute to dietary Ca requirements in humans (Grusak et al., 1996). Calcium (Ca) concentration in snap bean pods was influenced by genotypes, and environmental conditions such as heat units (temperature), rainfall, and soil Ca concentration (Quintana et al., 1999a). Lower temperature and soil moisture was associated with lower Ca concentration due to reduced root pressure (a means of water supply, which enables Ca transport up the plant to pods). Therefore, snap bean in dryland conditions may have lower Ca concentrations due to limited water supply. Other investigations show that Ca concentration in pods decreased with increasing pod diameter owing to low pod stomatal densities, and an increased relative humidity environment led to similar effects (Grusak and Pomper, 1999). Pod Ca concentration also varied with cultivar (Quintana et al. 1996; Miglioranza et al. 1997; Grusak and Pomper, 1999). Pods of certain genotypes appeared to have the ability to import Ca more efficiently than others. According to Favaro et al. (2007) there was a negative relationship between the N content in the pod and Ca concentration in the nutrient solution supplied to the snap bean plants.

According to Muchui et al. (2008) there were varietal differences among snap bean cultivars for pod number and yield. Differences were also observed among introduced and local cultivars of snap bean in Kenya with regard to yield potential and postharvest quality parameters such as pod length, curvature, seed size, and colour (Muchui et al., 2008).

Fruits and vegetables including snap bean are highly valued as sources of many vitamins and minerals. According to the California Department of Public Health's Network for Health California (2011) and Sotelo et al. (2003), snap bean provides more vitamin A, vitamin C and Ca, and higher fiber content but less starch and protein as compared to grain of common bean. However, Stolle-Smits et al. (1999) reported that protein content was decreased during subsequent pod development from a few days after flowering to senescence. Snap bean pods are very rich sources of dietary fiber and also contain an excellent level of Vitamin A and many health promoting flavonoids and poly-phenolic antioxidants. Bean are also good sources of vitamins and minerals (Silbernagel et al., 1991) like vitamin K, Ca (Grusak et al., 1996), magnesium and P (Moraghan, 1994).

Snap bean pods contain high concentration of protein, including the essential amino acid lysine. They also provide folic acid and some minerals. Protein concentration in pods of snap bean can be improved by soil N uptake by the plant (Ahmed et al., 2010). Research results indicated that protein and mineral concentration of snap bean pods were affected by cultural practices including N fertilizer and planting densities (Abubaker, 2008). Further, yield and quality of snap bean plant was significantly affected by biofertilizers (Salinas-Ramírez , 2011), and both macro and micro nutrients (Tantawy et al., 2009; Abdel-Mawgoud et al., 2011).

Planting season had a significant effect on most pod traits such as length, thickness, soluble solids content, tenderness, and string, and this effect varied markedly among environments (Hodges, 2004; Alhag and Hussein, 2014). Planting date and cultivar also had a significant impact on average pod yields of snap varieties (Hodges, 2004).

2.6 Snap bean under drought stress

Drought is a highly complex phenomenon that exerts deleterious impact on crop survival, productivity and quality of yield. The issue of drought needs a series of investigations for tackling problems associated with yield reduction and quality deterioration.

Snap bean cultivars displayed distinct responses to prolonged drought stress under field conditions. Photosynthetic rates, shoot extensions and leaf water status were related to soil water content (Kumar et al., 2006). Because bush type snap bean plants have shallow root systems, they need to have adequate moisture near the soil surface for shoot growth and development.

Snap bean is particularly susceptible to blossom drop under water stress, causing a split pod set (Taber, 2009). Flower drop can occur when soil moisture is less than 60% FC or the temperature is high with low relative humidity (Hodges, 1990; Barrios et al., 2005). The effects of drought stress vary depending on the frequency, duration and intensity of stress and stage of growth of the plant. Hodges (1990) reported that although the crop has an extensive root system, the plant is sensitive to moisture stress, especially during pod-set. Lack of water during vegetative and/or reproductive growth stages is one of the most limiting factors for bean growth (Boutraa and Sanders, 2001). Further research showed that excessive abortion of flowers and young pods occurred as a consequence of drought stress during pre-flowering and reproductive periods (Munoz-Perea et al., 2006). Drought stress also caused a gradual decline in the nodule host plant protein content and N_2 fixation (Ramos et al, 1999). Drought stress imposed during flowering reduced snap bean yield by 40% (Gunton and Evenson, 1980) to 50 % (Barrios et al., 2005).

Snap bean cultivars are variable for the response of drought which was extrapolated by malondialdehyde (MDA) production, a byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. Snap bean genotypes most affected by the stress produced more MDA than those that tolerated the stress (Yasar et al., 2010). The authors further pointed out that drought stress resulted in loss of chlorophyll. Water stress at flowering and pod filling stages reduced yield but not affect seed weight (Boutraa and Sanders, 2001).

According to Lazcano-Ferrat and Lovatt (1999), water-deficit stress on common bean significantly reduced leaf protein concentration (45-65%) and was accompanied by a decrease in vegetative growth (55%). The study also indicated that root dry weight was reduced due to moisture deficit for 18 days. Another report determined that leaf area index and biomass of common bean cultivars were adversely affected by drought treatments at vegetative and reproductive growth phases (Galegos and Shibata, 1989). Moreover, yield differences were also significant for water deficit treatments. Research on navy bean showed that moisture stress during pre-flowering and flowering stages reduced yield by 28 % and 24% respectively (Gunton and Evenson, 1980).

2.7 The role of growth regulators on drought responses

Drought stress reduced endogenous CK, indole-3-acetic acid (IAA), Zeatin and gibberellic acid (GA_3) concentration, and increased ABA concentration in common bean leaves (Figueiredo et al., 2008). Similarly, drought significantly decreased endogenous auxin (IAA), GA3 and CK levels while it increased ABA concentration (Sadeghipour and Aghaei, 2012). Hamayun et al. (2010) reported that drought induced by polyethylene glycol reduced endogenous gibberellin and increased ABA concentration in soybean. Increase in ABA concentration during stress deprived the chlorophyll biosynthesis pathway of the precursor pool which is a common precursor for both ABA and the energy harvesting chlorophyll molecule as well as xanthophyll, resulting in retarded growth (Sreenivasulu et al., 2012). Persistence of ABA may lead to pollen abortion through repression of cell wall invertase. In addition, high concentration of ABA during drought is correlated with inhibition of cell division in reproductive tissue and pod/seed abortion (Artlip et al., 1995).

Among plant growth regulators salicylic acid (SA), CK and ABA have been reported to play an important role in drought tolerance (Farooq et al. 2009b). Indole-3-acetic acid stimulated stomatal opening provided that both IAA and ABA were present. However, the stomatal opening was dependent on the relative concentration of each (Figueiredo et al., 2008). Another investigation indicated that CK was essential for recovery of the photosynthetic apparatus in bean after drought stress (Metwally et al., 1997). A review by Hare et al. (1997) indicated that CKs may assist in the complex but highly regulated coordination of growth and development during stress. Application of CK increased the production of proline (Thomas et al., 1992). Moisture stress also reduced CK production in plants (Seeley, 1990). Cytokinin can alleviate the stomatal closing effect of ABA and can partially inhibit accumulation of ABA induced by water stress (Pospisilova et al., 2005). A report indicated that application of natural products containing a significant amount of CK appeared to be associated with increased leaf CK concentration and greater drought resistance in some grasses (Zhang and Ervin, 2004). Application of supplementary CK prevented stomatal closure and reduced the effect of flooding stress in contrast to ABA application in tomato (*Solanum lycopersicum*) (Bradford, 1983). Applications of CK may reduce the impact of drought stress through osmotic adjustment, delay of stress-induced senescence, and reversion of early processes involved in leaf and fruit abscission

(Pospisilova et al., 2000). Abdullah and Ahmad (1990) reported that kinetin pretreatment lowered inhibition of protein content and nitrate reductase activity in salt stressed potato (*Solanum tuberosum*) plants. They found that levels of proline, reducing sugars and sodium were increased in different plant parts to maintain osmoregulation.

Application of exogenous SA to plants increased endogenous IAA, GA_3 and CK concentrations and reduced ABA thereby increased plant biomass production under drought stress in common bean (Sadeghipour and Aghaei, 2012). Exogenous applications of SA increased net photosynthetic rate and growth of chickpea (Hayat et al., 2012). Also SA increased dry mass and CO_2 assimilation rate due to improving the antioxidant defense system under drought stress in barley (*Hordeum vulgare*) (Habibi, 2012). El-Tayeb and Ahmed (2010) reported that application of SA improved total protein, total sugar and dry matter of wheat (*Triticum aestivum*) cultivars under drought stressed and unstressed conditions. Another study pointed out that SA application prevented the decrease in total biomass and seed vigor index frequently observed in drought stress in black cumin (*Bunium persicum*) (Kabiri et al., 2012). Salicylic acid induced stomatal closure, increased water use efficiency, increased chlorophyll content and intercellular CO_2 concentration in leaves of plants under drouht stress (Anosheh, 2012).

Yeast (*Saccharomyces cerevisiae*) is a source of plant regulating substances. Foliar application of active dry yeast significantly increased Fe, Zn and protein concentrations of cucumber fruit (*Cucumis sativus*) (Shehata et al., 2012). The same result was also observed in onion (*Allium cepa*) increased mineral nutrients including Fe, Zn, K, P and TSS (Fawzy et al., 2012). Yeast extract application also increased certain macro and micro nutrients such as nitrogen, P, K and Fe concentrations in green immature pea (*Pisum sativum*) pods (Asmaa et al., 2013). Foliar application of yeast extract significantly increased auxins and CK concentrations and reduced ABA concentration in the leaves of faba bean (El-Yazied and Mady, 2012). These authors indicated that yeast extract improved the reproductive characteristics of faba bean due to high levels of protein in the leaves and increased endogenous auxins and CK. A report by Mady (2009) indicated that yeast extract significantly reduced the shedding percentage of flowers and immature pod in faba bean. Abbas (2013) reported that application of biostimulants including yeast extract increased endogenous hormones such as auxin, CK, GA_3 and increased protein

synthesis that ultimately resulted in the enhanced performance of faba bean plants. Foliar application of yeast extract significantly increased the levels of endogenous CK, IAA and GA_3 concentration in snap bean leaves (El-Tohamy and and El-Greadly, 2007). In contrast, drought stress reduced endogenous cytokinins, IAA, Zeatin and GA_3 and increased ABA concentration in common bean leaves (Figueiredo et al., 2008). Similarly drought significantly decreased IAA, GA_3 and CK levels while increased ABA content (Sadeghipour and Aghaei, 2012). Yeast extract is a relatively inexpensive plant growth regulator, and its use in mitigating drought stress should be further explored as a management option for low-input snap bean production.

CHAPTER 3

3. Response of Snap Bean Cultivars to *Rhizobium* Inoculation under Dryland Agriculture

3.1 Abstract

Snap bean (*Phaseolus vulgaris* L.) is an economically important vegetable crop grown for both export and local markets in East Africa. Sustainable production requires relatively high nitrogen (N) inputs. However, it is not known if the use of rhizobial inoculants for enhanced biological dinitrogen fixation will provide adequate N early enough in the growing season to support optimal vegetative production required for bean produced as a vegetable crop. The objectives of this study were to test the use of rhizobia inoculation as an alternative N source for snap bean produced as a vegetable crop under rain fed conditions, and to identify suitable cultivars across contrasting environment for higher N_2 fixation and subsequent higher yield under dryland agriculture. The study was conducted in 2011 and 2012 during the main rainy season at three sites (Debre Zeit, Hawassa and Ziway) in Ethiopia. The treatments were a factorial combination of three N treatments (0 and 100 kg N ha^{-1}, and inoculation with *Rhizobium etli* [HB 429]) and eight snap bean cultivars. The experiment was also conducted under irrigation for one season in 2012 for comparison. Pod yield, pod number per plant, pod dry weight per plant, percentage of N derived from the atmosphere *(%Ndfa*) and nodulation measurements were assessed. Rhizobial inoculation and applied inorganic N increased the total yield of snap bean pod under rain fed conditions by 18 % and 42 %, respectively. Melkassa 1 was the most suitable cultivar for a reduced input production system due to its successful nodulation characteristics, greatest N_2 fixation levels and consistently good performance across locations under rain fed conditions. We concluded that N derived from biological N_2 fixation achieved through rhizobial inoculation of snap bean can support significant green pod yield improvement, particularly under rain fed conditions, and is suitable for low input vegetable production systems.

Key words: Snap bean; *Rhizobium*; Nitrogen fixation; ^{15}N

3.2 Introduction

Snap bean is a cultivar of common bean (*Phaseolus vulgaris* L.) from which immature pods are harvested and used as a vegetable for human consumption. Snap bean is characterized by succulent and flavorful pods (Stephen, 1998). Snap bean production in Eastern Africa has increased over time both for export and local market (FAOSTAT, 2014). In addition to large commercial vegetable farms that produce snap bean for export, snap bean is increasingly popular for small-scale vegetable producers for local markets. However, the burden of high input costs needs to be reduced for small-scale farmers in order to increase and sustain production, and maximize benefits of growing this cash crop. The use of rhizobia inoculants as an alternative N source, particularly for legume vegetables including snap bean, is less clear and currently is not widely practiced.

It is well-established that rhizobial inoculants are an effective means of enhancing N supply to legume crops, particularly in soils with low rhizobial populations. Under optimized environmental conditions, genetically superior genotypes of common bean that are nodulated with efficient *Rhizobium* strains are able to fix enough N to support grain yield (Bliss, 1993b; Thung and Rao, 1999; Kellman, 2008). The use of biological N fixation for sustainable grain crops production is, therefore, recommended (Safapour et al., 2011). However, previous studies typically target the role of N_2 fixation on dry seed yield of mature legume crops, which maximizes the N contribution of this beneficial association. In contrast, snap bean produced as a vegetable crop is harvested during early reproductive stages, long before the full benefits of season-long N_2 fixation have been realized. Reports are lacking whether N_2 fixation benefits are achieved early enough in the season to be beneficial in snap bean production. Pena-Cabriales et al. (1993) reported that nodulation and nitrogen fixation by common bean cultivars increased and reached a maximum during reproductive stages, and suggested that greater benefits to the plant by making N_2 fixation earlier could be achieved through earlier nodulation and a corresponding duration of fixation. Others similarly have reported maximum nodulation and N_2 fixation at pod formation (stage R7) (Muller et al., 1993; Araújo and Teixeira, 2000). The extent and continuation of N_2 fixation during pod development is cultivar dependent (Pena-Cabriales et al., 1993; Araujo and Teixeira, 2000). Others have observed N_2 fixation continues until the end of the growing season in legumes such as lentil (*Lens culinaris*) and significantly higher *%Ndfa* is

found in pods and seeds than in the leaves and stems (Van Kessel, 1994), suggesting that significant N_2 fixation may occur later in the growing season. Synchronization of high N_2 fixation activity and high N demand is crucial to derive the greatest benefits from biological N_2 fixation (Van Kessel, 1994; George and Singleton, 1992), and it is likely that the degree of synchronization varies among legume crop species (George and Singleton, 1992).

Some reports suggested that biologically fixed N is unable to satisfy the N demand for seed development of common bean because of senescence of nodules after flowering and before full demand occurs (Araújo et al., 2000). However, snap bean is harvested earlier, before full seed development. We have the opinion that the high N_2 fixation during the early pod filling stage (Muller et al., 1993; Araújo and Teixeira, 2000) may support the rapid development of the snap bean pods for the final vegetable market. Therefore, we hypothesized that pod yield increases can be achieved by sustaining N_2 fixation system until the commercial maturity of snap bean, and viable snap bean production can be realized using rhizobial inoculants in low input systems (rain fed production).

The objectives of this study were to test the use of rhizobia inoculation as an alternative N source for green pod snap bean production under rain fed conditions, and to identify suitable cultivars across contrasting environment for higher N_2 fixation and subsequent higher yield under low input dryland agriculture.

3.3 Materials and Methods

3.3.1 Experimental Sites

The study was conducted at three sites across different agro-ecologies in the Rift Valley of Ethiopia. The three locations were Debre Zeit ($8^o44'52''N$, $38^o05'53''E$) SolAgrow Private Limited Company farm; Hawassa ($7^o4'$ N, $38^o31'$ E) Hawassa University farm; and Ziway ($8^o00'$ N, $38^o45'E$) Ethioflora farm. Rain fall and temperature during the growing seasons, climate zones, altitude and soil physicochemical characteristics of each location are presented on Appendices 1 and 2. Debre Zeit has a tepid to cool sub-moist agro-ecology characterized by moderate temperature and a definitive rain fall pattern from July to September. It is situated at higher altitude in the transitional region of the Rift Valley and associated mountain ranges. The area is dominated by clay soils with higher copper and cation exchange capacity, and neutral pH.

Hawassa is in a hot to warm sub-moist humid agro-ecological zone with warmer temperatures especially during the dry season (February to April). It has a longer growing season and a relatively less defined pattern of rain fall during the growing season. It is a mid-highland area in the Rift Valley zone. The soil is loam characterized by slightly acidic pH and higher concentrations of micronutrients such as manganese, Fe and zinc (Zn). Ziway has a tepid to cool semi-arid agro-ecology with erratic rainfall and an unpredictable climate. The area is in the Rift Valley zone at mid-altitude. It has a warmer temperature particularly during the dry season. The soil is a sandy loam with very high pH and relatively high exchangeable sodium. Ziway is located at about a distance of 100 km each way in between Debre Zeit and Hawassa.

3.3.2 Experimental design and crop management

The experiment was conducted in 2011 and 2012 under rain fed conditions from June to September, and one season under irrigation during the dry season from February to April 2012 at the same three sites. The plots under rain fed conditions were seeded on June 27, July 6 and 19 in 2011 and on July 2, 4 and 1 in 2012 at Ziway, Debre Zeit and Hawassa, respectively. The experiment under irrigation was seeded on February 8, 9, and 12 at Ziway, Debre Zeit and Hawassa, respectively.

At each location and year, eight snap bean cultivars were tested against three N treatments combined in a randomized block in factorial combination of treatments with three replications. Among the eight cultivars, six (Andante, Boston, Contender Blue, Lomami, Paulista and Volta) were commercial cultivars currently under production in Ethiopia. The remaining two (Melkassa 1 and Melkassa 3) were local cultivars developed and recommended by the Melkassa Agricultural Research Center (MARC) for production in Ethiopia and are currently being grown by local farmers. The description of cultivars is presented on Appendix 3. The three N treatments were 0 and 100 kg N ha^{-1}, and *Rhizobium etli* (HB 429) inoculation. The rhizobium strain was developed by the National Soil Testing Center, Addis Ababa, Ethiopia and it is being used by local farmers for common bean grain production. The 100 kg N ha^{-1} is the average of commonly used N fertilizer rate used by commercial snap bean producers in Ethiopia. Seeds of snap bean cultivars for rhizobium inoculation treatment were coated with charcoal based rhizobium inoculum (*R. etli* [HB 429]). For this, fresh inoculum impregnated in charcoal was taken from National Soil Testing Centre, Addis Ababa, Ethiopia, in a week time of seeding

date. On the date of seeding, the snap bean seeds for inoculation were wetted with water with a spoon of sugar in it as a sticker solution. The charcoal base rhizobium inoculum was mixed thoroughly with seeds with sticker for proper coating. Then the coated seeds were dried under shade for approximately 20 -30 minutes and then seeded immediately. The detailed procedure is summarized in N2Africa (2014). The 100 kg N ha^{-1} treatment was apllied as a band application following the rows of the plot immediately after seeding in the form of granular urea.

Plot size was 2 m x 2.5 m. Each plot had five rows with 0.1 m between plants within each row and 0.5 m between rows with a row length of 2 m. The distance between adjacent blocks was 1.5 m. The two outer rows were considered border rows. All samples for data collection were taken from the three internal rows. Plant population was maintained by planting two seeds per hill and thinning to one plant upon appearance of trifoliate leaves. For the experiment under irrigation, furrow irrigation was applied starting from the date of seeding to the end of harvesting at four-day intervals based on evaporation demand and local experience for snap bean production. Plots were furrow irrigated until the soil ridges were saturated. For both rain fed and irrigated conditions the fungicide Mancozeb was applied at two-week intervals to control rust during vegetative growth until the pod formation stage. For irrigated conditions the fungicide Cortez was applied at the time when Fusarium stem rot was observed on growing snap bean plants. Manual weeding was used to control the weeds in the experimental plots.

Temperature and rainfall data were obtained from the nearest agricultural research center at each location (Appendix 1). Nitrogen fixation was assessed using the ^{15}N isotope dilution method and wheat was planted as a non-nodulating control plant for ^{15}N analysis under rain fed experiments (Tsai et al., 1993). In 2011, microplots measuring 0.75 m^2 were established within the treatment plots and a urea solution was applied at a rate of 10 kg N ha^{-1} labeled with 5 atom % ^{15}N excess subsequent to seedling emergence using a handheld watering can with a fine spray. The ^{15}N dilution was only successful at Debre Zeit; heavy rain at both Hawassa and Ziway shortly after ^{15}N application compromised the integrity of the ^{15}N in the microplots. The recommended rate of P fertilizer (21 kg ha^{-1} P) was applied in the form of triple super phosphate at each location during seeding time both under rain fed and irrigated trials.

In 2012, the ^{15}N natural abundance method was used to estimate biological N_2 fixation and wheat was used as the non-fixing reference crop for the rain fed experiments at all locations.

Wheat was planted in a row perpendicular to the inoculated snap bean plots 0.75 m from the snap bean row. Therefore, each plot had its own reference plants to minimize the impact of any natural ^{15}N variation in soils.

3.3.3 Measurements

3.3.3.1 Yield and yield components

Total pod yield was determined by the weight of the pods from three central rows of each plot and converted into t ha^{-1} at optimum maturity. All pods in a plant regardless of their quality were considered as total yield. Optimum maturity was considered when pods were firm with green young seeds, before seeds pushed out the pulp visibly. Generally, pods reached optimum maturity 19 – 25 days after flowering depending on cultivar and location. Pods from four randomly selected plants per plot were counted and the average was taken as pod number per plant. The dry weight of pods was determined after drying for 48 h at 70°C.

3.3.3.2 Nodulation and biological N_2 fixation

Three randomly selected plant samples were carefully uprooted at the flowering stage and nodules counted for total nodule number per plant root system. The diameters of all the nodules were measured using a caliper, and expressed as mean nodule diameter. Nodules were then dried in an oven for 24 h at 70^{o}C and weighed for determination of nodule dry weight per plant root system.

The percentage of N derived from the atmosphere *(%Ndfa*) was calculated by two methods, by ^{15}N isotopic dilution method in 2011 and by ^{15}N natural abundance method in 2012. For the ^{15}N isotopic dilution method, three plants were harvested from the central row of the micro-plot at the green pod mature stage (almost all of the pods on the plant reached maturity) in 2011 at Debre Zeit. Wheat plants were also harvested at the same time. Plant samples were dried in an oven at 50 ^{o}C to constant weight. The samples were ground and 20 g subsamples were taken for further analysis. The sub-samples were reground using a ball mill and very small portions (approximately 3 mg each sample) of the fine ground samples were pelleted into 6 x 8 mm tin caps. Samples were then analyzed using a Costech ECS4010 elemental analyzer (Costech

Analytical Technologies Inc., Valencia, California, USA) coupled to a Delta V mass spectrometer with a ConFlo IV interface (Thermo Scientific, Bremen, Germany).

%Ndfa was calculated using the formula [3.1] according to Hardarson and Danso (1993):

$$\%Ndfa = \left(1 - \frac{atom\%\ ^{15}N\ excess\ in\ snap\ bean}{atom\%\ ^{15}N\ excess\ in\ wheat}\right) \text{x } 100 \qquad [3.1]$$

Where "atom % excess" is the measure of the sample's ^{15}N content above the assumed atmospheric atom% ^{15}N value of 0.3663 (Mariotti, 1983).

When N_2 fixation was estimated using the ^{15}N natural abundant method in 2012, three snap bean plants were selected randomly from inoculated snap bean plots, accompanied by several wheat plants from the nearest wheat row. The above ground plant parts were harvested and analysed. The same procedures were followed with the ^{15}N isotopic dilution method mentioned above for sample preparation and analysis, and *%Ndfa* was calculated as follows:

$$\%Ndfa = \left(\frac{\delta^{15}N\ of\ wheat - \delta^{15}N\ of\ snap\ bean}{\delta^{15}N\ of\ wheat - B}\right) \text{x } 100 \qquad [3.2]$$

Where '$\delta^{15}N$' is:

$$\delta^{15}N\ (‰) = \left(\frac{atom\%\ ^{15}N\ sample - atom\%\ ^{15}N\ atmosphere}{atom\%\ ^{15}N\ atmosphere}\right) \text{x } 1000 \qquad [3.3]$$

and a B-value of -2 was assumed (Unkovich et al., 2008). The largest pool of N in the environment is atmospheric N_2 and it has a constant abundance of 0.3663 atom% ^{15}N (Mariotti, 1983).

Total fixed N was calculated using the formula [3.4] on a per hectare (ha) basis. In this experiment, only the above ground biomass was used to calculate total N.

$$\text{Total fixed N (kg ha}^{-1}) = \left(\frac{\%Ndfa}{100}\right) \text{ x Total N (kg ha}^{-1}) \qquad [3.4]$$

Where '*%Ndfa*' is obtained from the formula [3.2].

3.3.4 Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS software version 9.3 (SAS Institute Inc., 2012) to determine the analysis of variance (ANOVA). Nitrogen treatment, cultivar and location (agro-ecology) were considered as fixed effects, and replication (block), year and interactions with year were considered random. The covariance parameter estimate showed there was year by location by cultivar interaction for days to flowering and days to maturity. Therefore, a separate analysis was done for each year to identify the effect of location by cultivar interaction on these response variables for each year. The three locations were representatives of three different agro-ecologies or climate zones (Appendix 1). Assumptions of ANOVA for normality of distribution and homogeneity of variance were checked. Nodule number and nodule dry weight were log transformed based on the results of Levene's test for homogeneity of variance after inspecting the residuals. After analysis, these transformed values were back transformed for plotting graphs. Total yield from the irrigation experiment was analyzed separately. The DDFM=Kr option was considered for approximating the degree of freedom for means. Treatments were compared by LSD method. Significance was declared at $P \leq 0.05$. The *P*-values from mixed model ANOVA F-test Tables for the response variables is presented on Appendix 4.

3.4 Results

3.4.1 Total yield and yield components

Nitrogen treatment, cultivar and location significantly affected the total pod yield of snap bean both under rain fed and irrigated conditions. Application of 100 kg N ha^{-1} under the rain fed conditions resulted in the greatest pod yield followed by rhizobia inoculation with *R. etli* (HB 429) under the same conditions (Fig. 3.1a). Under irrigation, yields were similar between the unfertilized and inoculated treatments (Fig. 3.1a). For rain fed production, Melkassa 1 produced significantly greater yield than Andante, Contender Blue and Melkassa 3 (Fig. 3.1c). Volta produced significantly higher yield than Andante, Boston, Lomami and Melkassa 3 under irrigation (Fig. 3.1c). Andante was the lowest yielding cultivar under both conditions (Fig. 3.1c). The ranking of pod yields of Boston, Contender Blue and Lomami was not consistent under rain fed conditions or irrigation (Fig. 3.1c). Total yields from Hawassa and Debre Zeit were

significantly greater than the yield from Ziway under rain fed conditions (Fig. 3.1b). Under irrigation, total yield from Hawassa was the greatest of the other two locations (Fig. 3.1b).

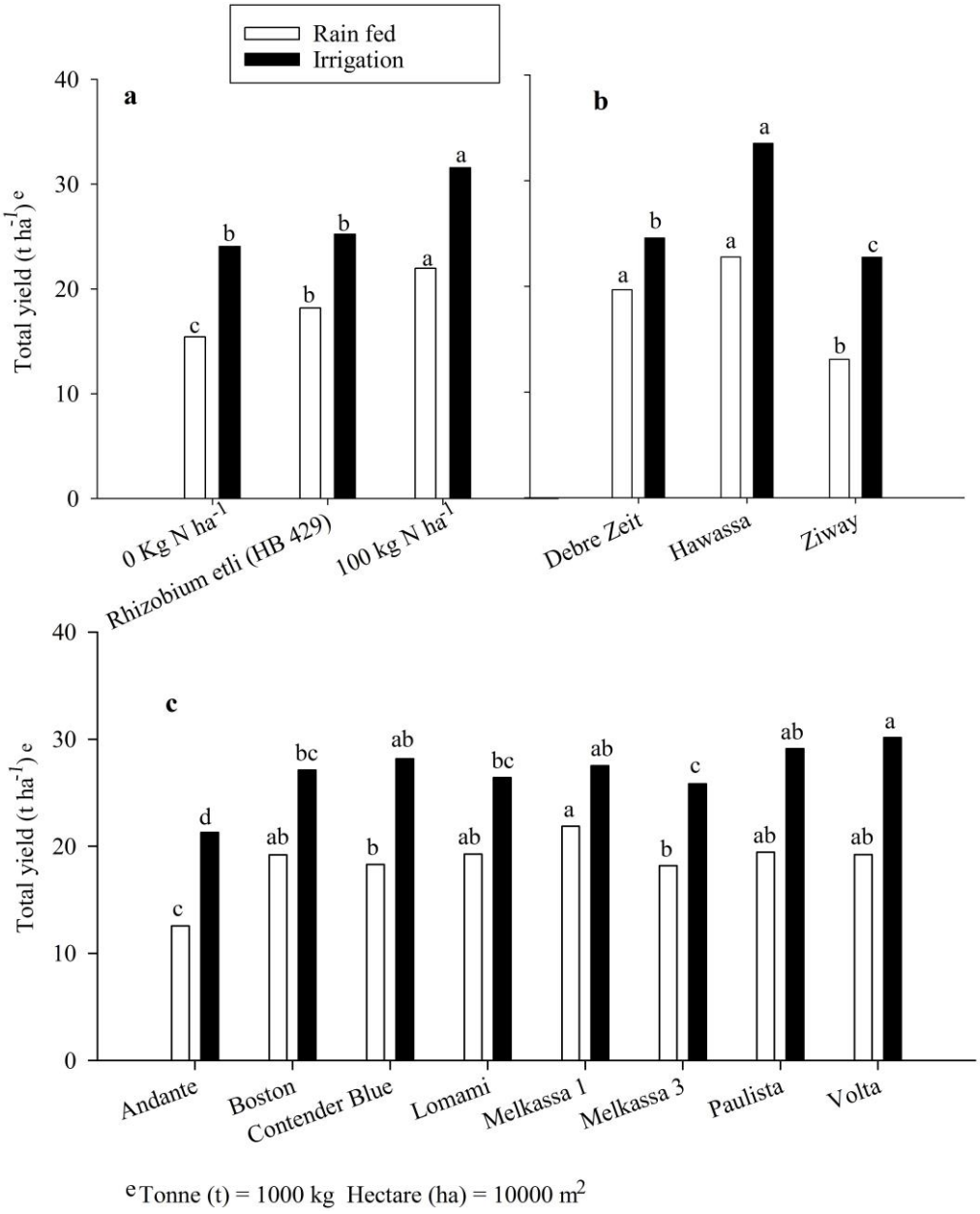


Fig. 3.1. Fresh pod yield of snap bean influenced by nitrogen treatment (a), location (b) and cultivar (c) under rain fed (2011 and 2012) and irrigation (2012) production systems. The same lower letter on the bars within panels (a), (b) or (c) indicates that means are not significantly different according to LSD at $P \leq 0.05$. Comparison should be made within rain fed and within irrigation groups separately.

Nitrogen treatment, cultivar, location and N treatment by location interaction significantly affected pod number per plant. Boston had the greatest number of pods per plant; however, it was comparable with the other cultivars, except Melkassa 1 and Melkassa 3 (Fig. 3.2b). The latter cultivar had the lowest numbers of pods per plant (Fig. 3.2b). For the N treatment by location interaction effect, the greatest number of pods per plant was observed when 100 kg N ha^{-1} was applied at Hawassa (Fig. 3.2a). The least number of pods per plant was produced at Ziway with 0 kg N ha^{-1}. Pod numbers in the rhizobium inoculation and 100 kg N ha^{-1} treatments were similar at Debre Zeit and Ziway; however, rhizobia inoculation was significantly different from the 0 kg N ha^{-1} at Debre Zeit (Fig. 3.2a). The 0 kg N ha^{-1} treatment at Hawassa was better than 100 kg N ha^{-1} application at Ziway and it was similar to 100 kg N ha^{-1} and rhizobia inoculation at Debre Zeit (Fig. 3.2a). None of the N treatments significantly affected pod numbers at Ziway (Fig. 3.2a).

Nitrogen treatment, cultivar and location significantly affected pod dry weight per plant. Application of 100 kg N ha^{-1} resulted in higher pod dry weight than 0 kg N ha^{-1} (Fig. 3.3). Pod dry weight in rhizobia inoculation treatments was not significantly different from either 0 or 100 kg N ha^{-1} (Fig. 3.3). Among cultivars, Melkassa 1 was greater in pod dry weight per plant than Andante, Contender Blue and Paulista (Fig. 3.2b). Andante had the lowest pod dry weight per plant (Fig. 3.2b). Andante and Melkassa 1 differed in days to flowering and days to maturity (Appendices 7 and 8). Melkassa cultivars were the greatest in leaf area index and numerically greater plant height than the commercial cultivars (Appendix 9). For the location effect, pod dry weight at Hawassa was comparable with Debre Zeit, but both were significantly higher than Ziway (Fig. 3.3). Cultivars at Hawassa and Debre Zeit had mostly comparable flowering and maturity date but they flowered earlier in 2011 and were earlier to mature both years at Ziway.

Nitrogen treatment and cultivar interacted with location to affect harvest index. All N treatments had similar harvest index at Debre Zeit. Rhizobium inoculation at Ziway and the control treatment both at Hawassa and Ziway had lower harvest index. Cultivars had inconsistent harvest index from location to location. Melkassa 1 had numerically the greatest harvest index at Debre Zeit and other cultivars generally had numerically better harvest index at Debre Zeit (Appendix 10).

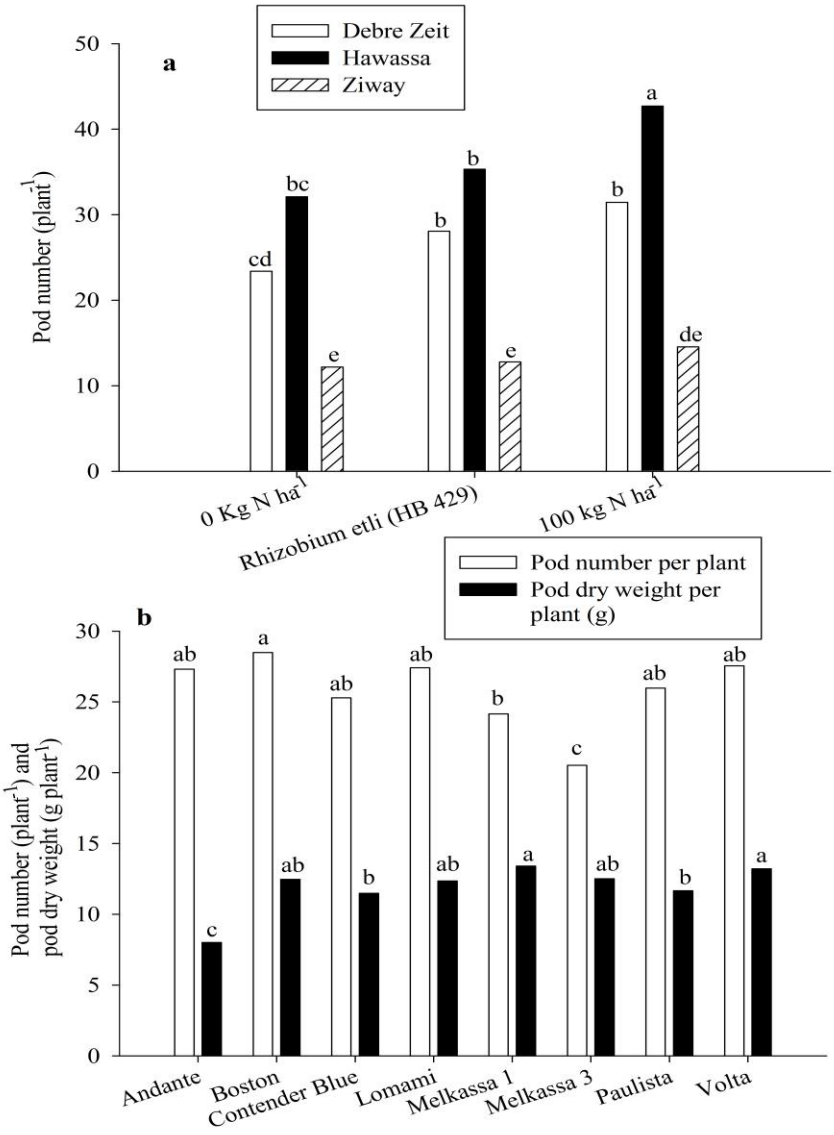


Fig. 3.2. Pod number and dry weight of snap bean as a result of nitrogen treatment by location interaction (a) and cultivar (b) under rain fed conditions (2011 and 2012). The same lower letter on the bars [among all bars in panel (a) and the same legend in panel (b)] indicates that means are not significantly different according to LSD at $P \leq 0.05$.

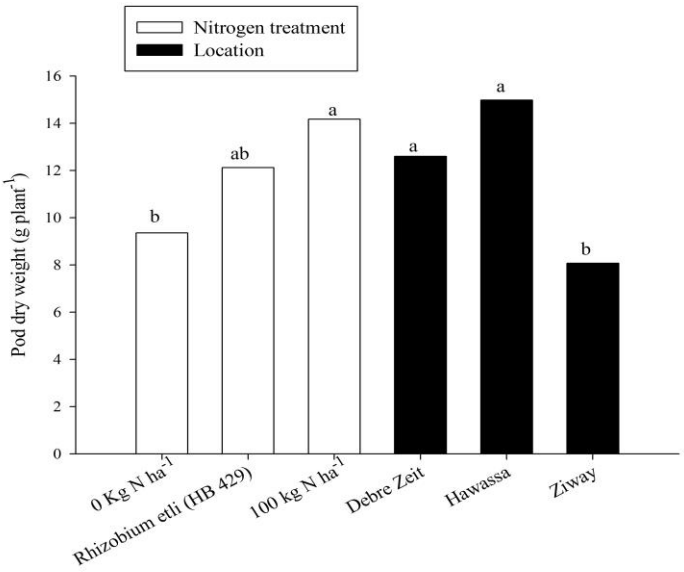


Fig. 3.3. Pod dry weight of snap bean affected by nitrogen treatment and location under rain fed conditions (2011 and 2012). The same lower letter on the bars with the same legend indicates that means are not significantly different according to LSD at $P \leq 0.05$.

3.4.2 Nodulation and biological N_2 fixation

Nodulation parameters were influenced in the study. Nitrogen treatment and cultivar significantly affected nodule number, total nodule dry weight and mean nodule diameter. Rhizobia inoculation resulted in the greatest nodule dry weight (Fig. 4a) and largest mean nodule diameter (Fig. 3.5a). This treatment also resulted in numerically higher nodule number. In contrast, N fertilizer application supressed these parameters. Variability across the cultivars was also observed for these nodulation parameters. Melkassa 1 produced the greatest nodule number of all cultivars except Lomami and Melkassa 3, and the greatest nodule dry weight except Lomami (Fig. 3.4b). Melkassa 1 had also the largest mean nodule diameter of all cultivars except Paulista and Volta (Fig. 3.5c). Andante had the lowest nodulation parameters (Fig. 3.4b, 3.5c). Location significantly affected only nodule diameter (Fig. 3.5b) out of the three nodule parameters. Larger mean nodule diameter was recorded from Debre Zeit compared to Ziway (Fig. 3.5b). Mean nodule diameter at Hawassa was not significantly different from either of the two other locations (Fig. 3.5b).

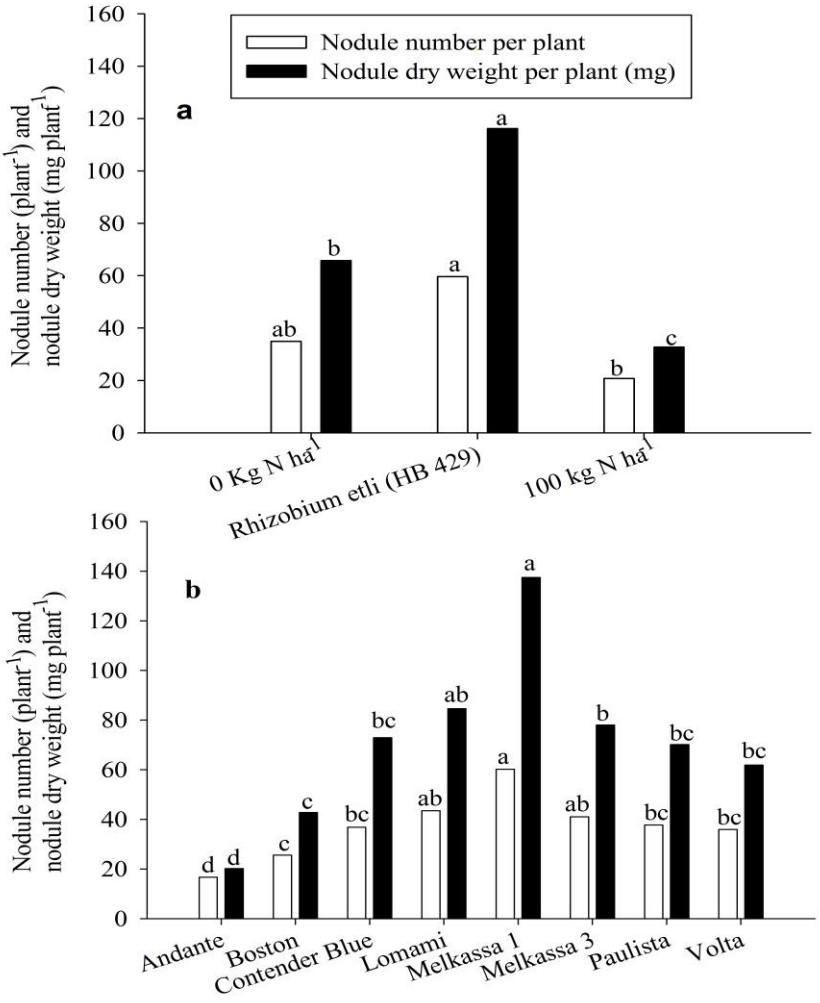


Fig. 3.4. Nodule number and dry weight of snap bean influenced by nitrogen treatment (a) and cultivar (b) under rain fed conditions (2011 and 2012). The same lower letter on the bars with the same legend within panels (a) and (b) indicates that means are not significantly different according to LSD at $P \leq 0.05$.

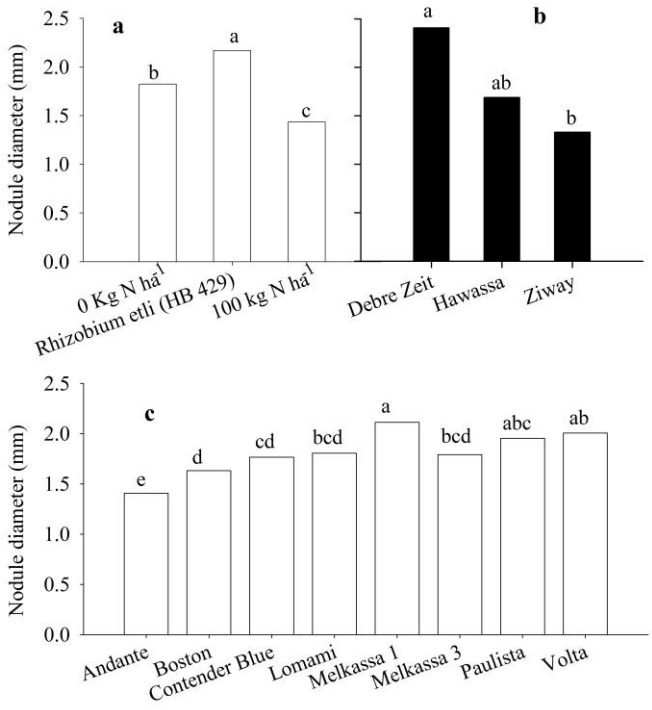


Fig. 3.5. Nodule diameter of snap bean influenced by nitrogen treatment (a) and cultivar (b) under rain fed conditions (2011 and 2012). The same lower letter on the bars within panels (a), (b) and (c) indicates that means are not significantly different according to LSD at $P \leq 0.05$.

There were significant differences among cultivars for *%Ndfa* which was determined by both ^{15}N dilution (only in Debre Zeit, 2011) and natural ^{15}N abundant methods (all three sites, 2012) (Fig. 3.6a). Results from both ^{15}N dilution and ^{15}N abundance methods indicated that Melkassa 1 had numerically higher *%Ndfa*. This cultivar was significantly different from Andante, Paulista and Volta in *%Ndfa* according to estimates using the natural ^{15}N abundance method and from all other cultivars according to the ^{15}N dilution method (Fig. 3.6a). According to the ^{15}N dilution method Contender Blue was the least effective of all cultivars in fixing N_2 (Fig. 3.6a). The results of this experiment also indicated that *%Ndfa* was highest at Debre Zeit followed by Ziway, and the least at Hawassa (Fig. 3.6b).

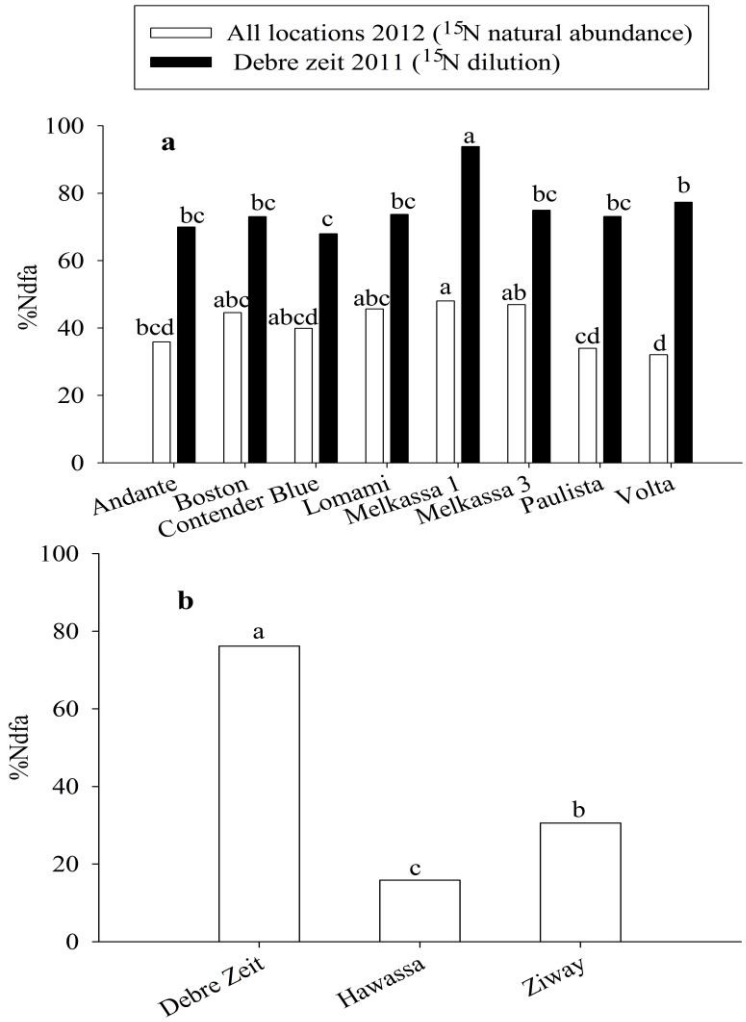


Fig. 3.6. Percentage of nitrogen derived from the atmosphere *(%Ndfa*) by snap bean as influenced by cultivar (a) and location (b) under rain fed conditions. The same lower letter on bars with the same legend within panels (a) and (b) indicates that the means are not significantly different according to LSD at $P \leq 0.05$.

Total fixed N ha^{-1} was significantly affected by cultivar and location. Melkassa 1 fixed the highest N ha^{-1} (Fig. 3.7a). Andante fixed the least N followed by Paulista (Fig. 3.7a). Boston, Lomami, Melkassa 3 and Volta fixed comparable amount of N with Melkassa 1 (3.7a). Andante, Contender Blue and Paulista fixed N which was significantly lower than Melkassa 1 (Fig. 3.7a). The greatest fixed N ha^{-1} was from Debre Zeit (Fig. 3.7b). Total fixed N was low at Hawassa and

Ziway (Fig.3.7b). Total fixed N followed similar pattern with *%Ndfa* with the exceptions of cultivars Contender Blue and Volta (Fig. 3.6a and 3.7a).

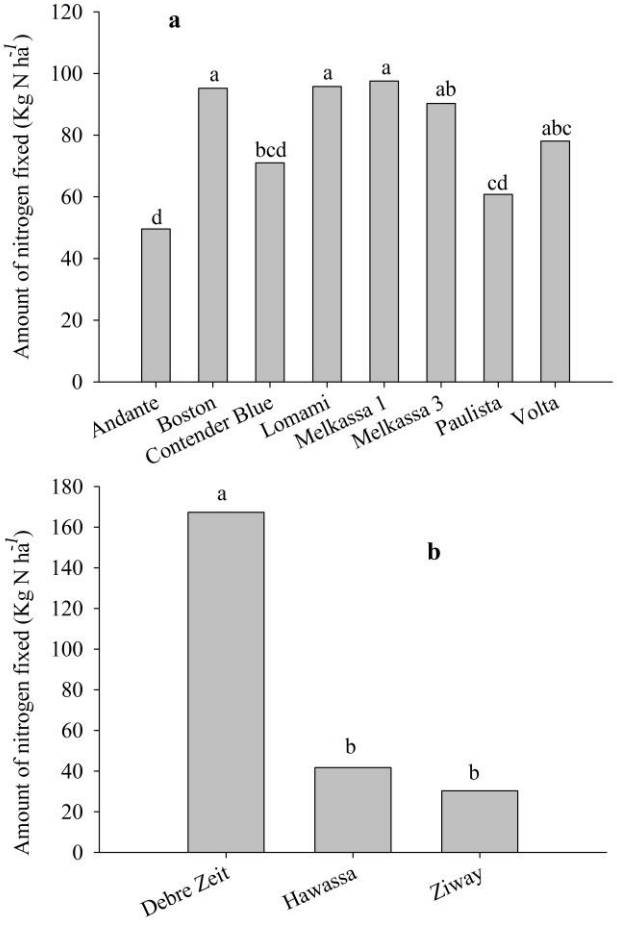


Fig. 3.7. Total nitrogen fixed by snap bean as influenced by cultivar (a) and location (b) under rain fed conditions (2012). The same lower letter on bars within panels (a) and (b) indicates that the means are not significantly different according to LSD at $P \leq 0.05$.

3.5 Discussion

This study demonstrated that N application consistently increased total yield, pod number per plant and pod dry weight of snap bean cultivars under both rain fed and irrigated conditions. Rhizobia inoculation increased the total yield and yield components of snap bean relative to an unfertilized and uninoculated control only under rain fed conditions. Increased yield (42%) due to applied inorganic N fertilizer was far greater than yield increases achieved by rhizobia inoculation (18%), suggesting that not all N requirements were met via biological N_2 fixation, or alternatively, that N_2 fixation taxed the energy requirements of the plant. Legumes use extra energy to fix N_2 from the air into useable forms (Pate et al., 1979; Kaschuk et al., 2009). Moreover, common bean has greater mineral N uptake efficiency as compared to when it is fixing N_2 (George and Singleton, 1992). This may indicate that early N demand could have been satisfied from the applied N source compared to N from fixation, so early N supply would have contributed to the rapid growth of leaves and accumulation of dry matter. Nitrogen fixation by legumes is also affected by a number of edaphic, climatic and biotic factors (Mulongoy, 1992) that may reduce its effectiveness when compared to applied N.

The greater pod number was obtained by 100 kg N ha^{-1} at Hawassa. Application of 100 kg N ha^{-1} and rhizobia inoculation at Debre Zeit resulted in similar pod numbers compared to rhizobia inoculation and the control at Hawassa. Within each location, rhizobium inoculation was significantly different from the control only at Debre Zeit. This result clearly indicated that rhizobia inoculation was most effective at Debre Zeit. Hawassa was the most suitable area for increasing pod number of snap bean. A greater number of pods lead to higher yield as these two traits have direct association (Araújo et al., 2012).

Although applied N resulted in the highest pod yield and yield components of snap bean, rhizobial inoculation also improved the vegetable yield of snap bean (green pod yield) as compared to the control treatment under rain fed conditions. This result suggests that pod yield increases can be achieved by a N_2 fixation system sustained until commercial maturity of snap bean (green pod), and viable snap bean production can be realized using rhizobial inoculant in low input systems.

Inoculation with *R. etli* (HB 429) increased nodule number, nodule dry weight and diameter of nodules, where as, N application suppressed these parameters under rain fed conditions. The current result was similar with previous reports (Da Silva et al., 1993; Fan et al., 1997; Giller et al., 1998; Otieno et al., 2009; Amba et al., 2013) conducted on common bean and other grain legumes. Nodule numbers under 0 kg N ha^{-1} were comparable to the inoculated treatment. This may be due to higher number of indigenous rhizobia in the research site which usually resulted in small ineffective nodules as reflected by nodule diameter and dry weight.

Yield variability was observed among the current cultivars. Genetic variability affected the performance of common bean cultivars in terms of shoot biomass and yield (Mourice and Tryphone, 2012). Our result indicated that Melkassa 1 was the best cultivar to grow under rain fed conditions. This cultivar exhibited substantial N_2 fixation and greatest yield compared to other cultivars under rain fed conditions. Melkassa 1 also had the greatest pod dry weight at fresh pod maturity stage and was among the top cultivars for number of pods produced under rain fed conditions.

This study demonstrated that the cultivar Melkassa 1 has the potential to produce large numbers of nodules with larger nodule sizes resulting in greater overall nodule dry weights. Moreover, Melkassa 1 achieved relatively high levels of *%Ndfa* which was determined by both ^{15}N dilution method from Debre Zeit site in 2011 and ^{15}N natural abundance method across all locations in 2012. Most importantly Melkassa 1 also fixed the highest N ha^{-1}. Previous investigations explained that effectiveness of N_2 fixation by rhizobia and further conversion into yield is affected by genotype in field bean (Bildirici and Yilmaz, 2005; Bliss, 1993a; Diouf et al., 2008; Sadowsky et al., 1988). The current result further indicated the occurrence of variations among snap bean cultivars for effective N_2 fixation. The data for *%Ndfa*, which was determined by the ^{15}N dilution method, were obtained only from a single location (Debre Zeit) in 2011. The nodulation and N_2 fixation data showed that higher N_2 fixation occurred at Debre Zeit as compared to the other two locations. The data for *%Ndfa*, which were determined by the natural abundant method, were from all three locations in 2012. The lower *%Ndfa* from Hawassa and Ziway may have lowered the average *%Ndfa* in 2012. This may be the main reason for the discrepancy between ^{15}N dilution and natural abundance methods on *%Ndfa* values in the current experiment.

The greater N_2 fixation response in this experiment compared to previous reports on common bean (Musandu and Joshua, 2001; Otieno et al., 2009) may be due to the effectiveness of the strain we used, the response of the cultivars, appropriate site selection or suitable growing season during experiments. Many other factors including improved crop management may also contribute to a favorable outcome. Moreover, several reports demonstrated that significant improvement of yield and yield components in common bean was due to N_2 fixation (Da Silva et al., 1993; Daba and Haile, 2002; Bildirici and Yilmaz, 2005; Cardoso et al., 2007; Kellman, 2008). The ineffectiveness of rhizobia inoculation in increasing yield under irrigation may be due to higher soil temperature during the growing season as previously reported by Piha and Munns (1987).

Nodule formation by snap bean was absent in almost all plants within plots at Hawassa and Ziway under irrigation. At Debre Zeit, nodulation was observed even under irrigation but it was statistically similar among other N treatments. We suggest that higher temperatures during the irrigated experiment, which took place during the dry season (February to April), may restrict nodulation. However, the complete absence of nodules observed in most plots at Hawassa and Ziway during the irrigation experiment requires further investigation.

Our study also demonstrated that *%Ndfa* and total fixed N ha^{-1} were highest at Debre Zeit which was also reflected in the number of pods per plant from interactions between N treatment and location. In addition to other factors (Bordeleau and Prevost, 1994; Toro, 1996), higher *%Ndfa* and total fixed N ha^{-1} at Debre Zeit may be due to greater copper concentration in the soil that contributed to the effectiveness of N_2 fixation (Snowball et al., 1980; Seliga, 1998). Nodule size was greater at Hawassa than at Ziway but the reverse was observed for *%Ndfa* for these two locations. This may indicate that the presence of nodules and their average size may not necessarily guarantee effective N_2 fixation. However, large biomass at Hawassa may contribute to high total N leading to high fixed N compared to at Ziway.

3.6 Conclusions

Snap bean pod yield improvement can be achieved by N_2 fixation system sustained until the commercial maturity of snap bean, and viable snap bean production can be realized using rhizobial inoculant in low input systems. Rhizobia inoculation was not as effective as high rates of inorganic N fertilizer, but still remains a viable and potentially less expensive alternative for improving the pod yield of snap bean under rain fed conditions.

The demonstration that snap bean can be successfully produced without irrigation is an important finding for farmers with no irrigation infrastructure. Melkassa 1 was the highest yielding cultivar and most suitable for a reduced input production system due to its successful nodulation character, highest N_2 fixation and consistently high performance across locations under rain fed conditions. Conditions at Debre Zeit were the most conducive for supporting biological N_2 fixation for snap bean production. This shows potentially the best possible use of rhizobial inoculant as N source in areas at other parts of the world with similar agro-ecological characteristics of Debre Zeit. Ziway, which was characterized by a semi-arid environment with unpredictable rain fall, was a less suitable area for snap bean production.

3.7 Prologue to chapter 4

In chapter 3 the use of rhizobial inoculation on total yield improvement of snap bean cultivars and N_2 fixation responses of the cultivars under contrasting environment (agro-ecology) were studied. It was demonstrated that successful nodulation and N_2 fixation can be achieved by snap bean cultivars to support acceptable pod yield. The nodulation and N_2 fixation data showed that cultivars vary in N_2 fixation and Melkassa 1 was the greatest N_2 fixer. For adequate N_2 fixation, agro-ecological variation plays a significant role. It is therefore important to consider cultivars with greater N_2 fixation potential and appropriate agro-ecology in order to get maximum benefits from N_2 fixation to improve yield of snap bean. However, the effect of rhizobial inoculation, cultivar and agro-ecological differences on the pod physical qualities and nutrient concentrations need further investigation. A study in chapter 4 was a continuation of chapter 3 focused on the effect of rhizobial inoculation, cultivar and agro-ecological variations on snap bean marketable pod yield, quality and nutrient concentrations.

CHAPTER 4

4. Pod Quality of Snap Bean Cultivars in Response to Nitrogen Treatment and Agro-ecology under Dryland Agriculture

4.1 Abstract

Snap bean (*Phaseolus vulgaris* L.) production under rain fed conditions by resource limited farmers can be increased if suitable management practices are available to produce export quality pods. The objectives were (1) to assess the possible use of rhizobium inoculation as a source of nitrogen (N) to produce quality snap bean pods relative to the common rate of synthetic N fertilizer and a control treatment without N; (2) to evaluate cultivars of snap bean for physical pod quality and high nutrient concentrations; and (3) to determine marketable yield, pod physical qualities, and nutrient concentrations of snap bean under contrasting environments. The study was conducted in 2011 and 2012 under rain fed conditions at three locations (Debre Zeit, Hawassa, Ziway) representing distinct agro-ecologies in Ethiopia. Three N treatments (0 kg N ha^{-1}, inoculation with *Rhizobium etli* [HB 429], 100 kg N ha^{-1}) were combined with eight cultivars factorially arranged in a randomized complete block design replicated three times. Marketable pod yield, other physical qualities and nutrient concentrations of pods were recorded. Applied N and rhizobium inoculant increased marketable yield by 43 and 18%, respectively. Melkassa 1 had numerically the greatest marketable yield but performed lower in other pod physical qualities such as pod texture and appearance. Cultivars interacted with locations to affect pod traits including total soluble solids and concentrations of protein, calcium, and potassium. Snap bean pods produced at Debre Zeit and Hawassa were similar in marketable yield and several other traits. Zinc concentration in pods was greatest at Hawassa. Ziway, with a more arid climate and soil pH above 8.0, was the least favorable location for snap bean production. In conclusion, viable production of marketable quality snap bean pods can be achieved by using rhizobial inoculation as N source particularly for resource limited farmers.

Key words: Snap bean; Cultivars; Quality; Nitrogen; Rhizobium

4.2 Introduction

Snap bean cultivars are specific type (or class) of common bean (*Phaseolus vulgaris* L.) grown for their green pods used as vegetables which serve as an important source of protein. The pod physical quality of snap bean is a combination of appearance and its physical condition. Acceptable snap bean quality includes well-formed and straight pods, and pods should be bright in color with a fresh appearance, free of defects, tender (not tough or stringy) and firm (Cantwell and Suslow, 1998).

The quality of snap bean pods can also be expressed in terms of nutrient concentrations because they are important in human nutrition. Over two billion people are affected by micronutrient malnutrition in the developing world (Cakmak et al., 2010). Iron (Fe) and Zn (Zn) deficiencies predominate affecting preschool children that impair physical growth and mental development (Fe), hamper growth and development (Zn), and destroy the immune system (Zn) (Cakmak et al., 2010). Micronutrient malnutrition and hunger can be alleviated through common bean nutrition because pods are rich in quality protein, fibre, micronutrients such as Fe, Zn and vitamin A (Ugen et al., 2012). Common bean also contains high protein, including the essential amino acid lysine (Baudoin and Maquet 1999). Although information is lacking for direct comparison, independent research results showed that snap bean immature pods on a dry weight basis contain a similar range of protein concentration as dry seeds of common bean (Abubaker, 2008; Pereira et al., 2009). But the high moisture content in fresh pods dilutes the protein concentration on fresh weight basis as vegetable. Snap bean also possesses high bioavailable calcium (Ca) when compared to other foods (Quintana et al., 1999a). Protein concentration in pods of snap bean can be improved by soil N uptake by the plant (Ahmed et al., 2010). Protein and mineral concentrations of snap bean pods can be affected by cultural practices including N fertilizer (Abubaker, 2008). Further, yield and quality of snap bean plant were significantly improved by organic fertilizers (Salinas-Ramírez et al., 2011), and both macro and micro nutrient application (Tantawy et al., 2009; Abdel-Mawgoud et al., 2011).

The use of synthetic N for improving pod quality of snap bean is well documented and extensively studied. However, dependency on synthetic fertilizers needs to be minimized because greater application rates are constrained by increased fertilizer prices and they release greenhouse gases along with field losses due to inefficacy of application (Reid et al., 2011;

Ferguson et al., 2010; Ferguson, 2013). Extensive reports are available on the realization of rhizobium inoculations in increasing yields of chickpea (*Cicer arietinum* L.; Bhuiyan et al., 2008), soybean (*Glycine max Merr.;* Sall and Sinclair, 1991), common bean (Bildirici and Yilmaz, 2005; Otieno et al., 2009), and many other legumes. However, the use of rhizobium inoculant in improving the quality of snap bean is lacking. Rubatzky and Yamaguchi (1997) have questioned the use of rhizobium inoculation for vegetable legume production because inoculation may not produce adequate N_2 fixation sufficiently early in the season to support a vegetable pod harvest.

The productivity and quality of a given crop species or cultivar can be determined by crop management and agro-meteorological variables such as rainfall, soil and temperature (Dapaah, 1997; Hoogenboom, 2000). Individual legume species or cultivars often require specific ecological niches for maximum production (Masaya and White, 1991) that should not be ignored when considering site suitability whether at local or national levels (Valentine and Matthew, 1999). Knowledge of the developmental and environmental factors contributing to yield and quality variation is therefore required to maximize yield and quality of agricultural crops. Yield variation was observed among different common bean genotypes in east Africa (Tanzania) among different locations (Giller et al., 1998). Nutrient concentration in seeds of common bean was also influenced by genotype (Nchimbi-Msolla and Tryphone, 2010; Prolla et al., 2010; Beebe et al., 2000; Gregorio, 2002) and environment (Quintana et al., 1999b; Nchimbi-Msolla and Tryphone, 2010). However, studies are lacking on the influence of climate zones (environment) on the physical and nutrient concentrations of snap bean pods. The interactive effect of environment and cultivar differences on pod quality of snap bean also needs investigation.

The current chapter is a continuation of the previous chapter focused on the effect of rhizobial inoculation on snap bean marketable pod yield, quality and nutrient concentrations.Therefore our study in the current chapter aimed to assess the possible use of rhizobium inoculation as a source of N to produce quality snap bean pods relative to the common dose of synthetic N fertilizer and the control treatment without N. The second objective was to evaluate cultivars of snap bean for physical pod qualities and nutrient concentrations in the pods. The third objective was to determine marketable yield, pod physical qualities, and nutrient concentrations of snap bean under contrasting environments.

4.3 Materials and methods

4.3.1 Site characteristics

This experiment was conducted at three locations found in different agro-ecological zones in Ethiopia. The three locations were Debre Zeit ($8^{o}44'52''N$, $38^{o}05'53''E$) SolAgrow Private Limited Company farm; Hawassa ($7^{o}4'$ N, $38^{o}31'$ E) Hawassa University farm; and Ziway ($8^{o}00'$ N, $38^{o}45'E$) Ethioflora farm. Complete descriptions of the climate, agro-meteorological variables and soil characteristics of the three sites are presented in Chapter 3.3.1 and Appendices 1 and 2.

4.3.2 Experimental design and crop management

The field experiments were conducted under rain fed conditions in 2011 and 2012 during the main rainy season from June to September (the normal planting season). The detailed experimental design and crop management is explained in Chapter 3.3.2.

4.3.3 Measurements

4.3.3.1 Pod marketable yield and other physical qualities

Pods at optimum maturity (chapter 3.3.3.1 and Appendix 8) were harvested and the weights of marketable pods (i.e. only defect free, acceptable quality pods) were calculated as t ha^{-1}. The length and diameter of pods from four randomly selected sample plants were measured with a tape measure and sieve, respectively. Pod curvature was calculated by measuring the actual length of the pod and the shortest distance from the two ends of the pod. The ratio of the later to the former shows the extent of the curvature of the pod.

Texture and appearance were scaled using a visual rating scale modified from Martinez et al. (1995) and Proulx et al. (2010). Pod texture and appearance were rated by five experts who grade and pack snap bean for export markets. The surface quality of the pods was expressed as pod texture. Pod texture scales were: 1= very fine (extremely smooth surface); 2 = fine (smooth surface); 3 = reasonably fine (moderately smooth surface); 4 = coarse (rough surface); 5 = very coarse (very rough surface). Pod appearance was expressed as the overall look of the pods which is a combination of different expresions on the pod. Pod appearance was scaled as: 1 = excellent (field fresh, bright, straight, extremely tender and firm, snaps very easily, uniform); 2 = good

(field fresh, bright, slightly curved, tender and firm, snap easily, snap easily, slightly rippled); 3 = acceptable (moderately field fresh, moderate bright, moderate curved, tender but less firm, moderately rippled); 4 = poor (less fresh, green but not bright, curved, bent easily not snap, rippled, some blemish on surface); 5 = rejected (dull green, very curved, not snap, very rippled, blemish on surface, defective with disease and insect bit). For titratable acidity (TA), aliquots (10.0 g) of juice were diluted with 50 mL distilled water and acidity determined by titration with 0.1 N NaOH end point (pink color). The results were converted to percentage malic acid, which is the main organic acid in snap bean (Martinez et al., 1995; Proulx et al., 2010) using the formula [4.1] of Proulx et al. (2010),

$$TA = \frac{\text{mL NaOH} * 0.1\ \text{N} * 0.067\ \text{meq}}{10.0\ \text{g}} \times 100 \qquad [4.1]$$

Where TA= Titratable acidity, mL= milliliter, NaOH= Sodium hydroxide, N=Normal (normality of NaOH), meq= milli-equivalent (molecular weight of malic acid = 67), g=gram (juice).

At optimum green pod maturity stage, TSS of pods was measured using a hand-held refractometer for Brix (TBT, RHB0-80, Jiangsu, China).

4.3.3.2 Nutrient concentrations

Total N and P in green pods of snap bean were measured by a sulfuric acid-hydrogen peroxide digestion using a temperature-controlled digestion block (Thomas et al. 1967), followed by determination of total N and phosphate concentration in the digest using automated colorimetry (Technicon Instruments Corporation, New York, USA) (Wall et al., 1975; Watanabe and Olsen ,1965). Protein concentration was estimated by multiplying the N value by 6.25 (Imran et al., 2008). Protein content of the pods was derived from protein concentration multiplied by pod dry weight per unit area. Nutrient concentrations were determined on dry weight basis. To convert the protein concentration in to fresh weight basis, fresh snap bean pods were dried in an oven for 48 h at 70 ^{o}C. The mosture content of the fresh pod was 92%. A conversion factor (11.93) was calculated that a gram of pod dry weight (DW) has 11.93 g of pod fresh weight (FW) i.e.

$$\text{Protein cncentration (\% in FW)} = \left(\frac{\text{\% Protein cencentration in DW}}{11.93}\right) \qquad [4.2]$$

Zinc and Fe concentrations were analyzed on the NovAA®330 atomic absorption spectrometer (AAS) (Analytikjena, Jena, Germany) using an air/acetylene flame. Calcium and K concentrations were analyzed using the same AAS using nitrous oxide as the oxidant for the acetylene.

4.3.4 Statistical analysis

Data were analysed using PROC MIXED procedure of 9.3 SAS software (SAS Institute Inc. 2012). The assumptions of ANOVA for normality of distribution and homogeneity of variance were checked. The two years data were combined for analysis. The covariance parameter estimate showed there was year by location by cultivar interaction for protein and Ca concentrations, and protein content. Therefore, a separate analysis was done for each year to identify the effect of location by cultivar interaction on these response variables at each year. The TSS and acidity data were available only in 2012. Nitrogen treatments, cultivar and locations (agro-ecology) were considered as fixed effects. Year, block nested in year, the interaction of each of main plot factors (N treatment, cultivar and location) with year and the two-way and three-way interactions of main plot factors with year were considered as random. The non-significant covariance parameters were eliminated starting from the higher level of interaction from the model according to AIC values to simplify the model for better model fit (Littell et al., 2005). Means were separated according Fisher's protected LSD, at $P \leq 0.05$. The *P*-values from mixed model ANOVA F-test Tables for the response variables are presented on Appendices 5 and 6.The three locations were representatives of three distinct agro-ecologies or climate zones (Appendix 1). Therefore, agro-ecology was a fixed effect and presented as "location".

4.4 Results

4.4.1 Marketable pod yield and other physical qualities

Nitrogen treatment, cultivar and location significantly affected marketable yield. The highest marketable pod yield was produced using 100 kg N ha^{-1} (Table 4.1). Rhizobium inoculation resulted in significantly higher marketable pod yield than the Zero N (Table 4.1). The greatest and the least marketable yields were produced by Melkassa 1 and Andante, respectively (Table 4.1). Hawassa and Debre Zeit were found to be suitable areas to produce snap bean, both with significantly higher marketable yield than Ziway (Table 4.1).

Cultivar had significant effect on pod length and pod diameter of snap bean. However, N treatments and locations had no effect on pod length and diameter. Melkassa 3 produced the longest pods of all cultivars (Table 4.1). Among the commercial cultivars, Volta produced longer pods than Andante, Contender Blue and Lomami. In contrast, Andante produced the shortest pods of all cultivars followed by Contender Blue (Table 4.1). Melkassa 1 produced the largest pod diameter followed by Melkassa 3 (Table 4.1). Volta produced the larger pod diameter among the commercial cultivars (Table 4.1). Pods from Volta were similar in diameter to pods from Contender Blue, Lomami and Paulista (Table 4.1). On the other hand, Andante produced the smallest pod diameter of all cultivars (Table 4.1).

Cultivar had a significant effect on texture of snap bean pods. But N treatment and location had no effect on the texture of pods. The interactions of N treatment by cultivar and N treatment by location significantly affected the texture of snap bean pods. Commercial cultivars generally also had better pod texture than Melkassa cultivars (Table 4.1). Commercial cultivars had smooth and uniform pod texture in contrast to pods from Melkassa cultivars which were rough and lacked uniformity. The cultivar differences in pod texture were enhanced by N as seen in the interaction of N treatment by cultivar (Table 4.2). The best textures observed in Contender Blue, Lomami, Paulista and Volta were all obtained under 100 kg N ha^{-1} application apart from Andante and Boston which already at best regardless of N treatment (Table 4.2). Nitrogen application also improved the texture of Melkassa 1 (Table 4.2). The results from the N treatment by location interaction showed that N application at Ziway resulted in better texture than the control at Debre Zeit (Table 4.2). Generally, N application had some influence on the texture of snap bean pods at all locations (Table 4.2).

Nitrogen treatment and cultivar had significant effect on the appearance of snap bean pods but location did not. The interaction of N treatment by cultivar also significantly affected snap bean pod appearance. An excellent appearance of snap bean pods resulted from 100 kg N ha^{-1} (Table 4.1). Rhizobium inoculation also improved the appearance of snap bean pods compared to the control (Table 4.1). Commercial cultivars produced the best pod appearance (Table 4.1). The appearance of Melkassa cultivars were in the acceptable range but not as good as commercial cultivars (Table 4.1).

For the N by cultivar interaction, the appearances of all cultivars were affected by 100 kg N ha^{-1} (Table 4.2). All commercial cultivars were at their best appearance level at 100 kg N ha^{-1} (Table 4.2). Applied N fertilizer resulted in better appearance than rhizobium inoculation for all cultivars except Melkassa 1 (Table 4.2). Appearances of both Melkassa cultivars instead were most improved by rhizobium inoculation compared to the control (Table 4.2). There were no differences between rhizobium inoculation and the Zero N control for pod appearance of commercial cultivars (Table 4.2). Rhizobium inoculation was more effective in improving the appearance of Melkassa cultivars.

Nitrogen treatment, cultivar and locations significantly affected TA of snap bean pods. First, N application and rhizobium inoculation increased TA of snap bean pods (Table 4.1). For the cultivar response, Lomami had higher pod TA, but was similar to Andante, Boston, Contender Blue, Melkassa 3 and Volta (Table 4.1). Numerically the least percentage of TA came from Melkassa 1which was statistically similar to Melkassa 3 and Paulista (Table 4.1). Hawassa and Debre Zeit resulted in a higher percentage of TA in snap bean pods compared to pods from Ziway (Table 4.1).

Cultivar significantly affected the TSS of snap bean pods. Nitrogen treatment and location had no effect on TSS of pods. The location by cultivar interaction had also a significant effect on the TSS of snap bean pods. Within cultivars, there was a sliding range of TSS, with Melkassa 3, Paulista and Volta having greater TSS than Andante and Boston (Table 4.1). For the interaction, Paulista at Hawassa had numerically the highest TSS but it differed significantly only from Boston at Hawassa, and from Andante and Contender Blue at Ziway (Table 4.2).

Table 4.1. Pod marketable yield, length, diameter, texture, appearance, titratable acidity (TA) and total soluble solids (TSS) of snap bean cultivars (2011 and 2012)

	Marketable yield (t ha^{-1})	Pod length (mm)	Pod diameter (mm)	Texture (1-5)†	Appearance (1-5)‡	TA (%)§	TSS (^{o}Brix)§
Nitrogen treatment							
0 kg N ha^{-1}	14.39c	120.2	7.38	1.98	2.13a	0.0701b	5.46
Rhizobium etli (HB 429)	16.92b	122.0	7.49	1.91	1.81b	0.0747a	5.50
100 kg N ha^{-1}	20.54a	125.0	7.56	1.21	1.21c	0.0769a	5.54
Cultivar							
Andante	11.70c	106.4e	6.01e	1.42b	1.57b	0.0765a	5.44b
Boston	17.94b	123.1bc	7.11d	1.44b	1.54b	0.0768a	5.41b
Contender Blue	16.94b	112.8d	7.38cd	1.55b	1.56b	0.0747ab	5.47ab
Lomami	18.14ab	122.7c	7.44cd	1.59b	1.63b	0.0775a	5.51ab
Melkassa 1	20.60a	125.8bc	8.68a	2.26a	2.26a	0.0668c	5.49ab
Melkassa 3	16.95b	133.8a	8.32b	2.15a	2.15a	0.0726abc	5.56a
Paulista	17.98b	126.5bc	7.36cd	1.57b	1.5b	0.0700bc	5.57a
Volta	18.00b	128.1b	7.48c	1.61b	1.54b	0.0763a	5.56a
Location							
Debre Zeit	18.45a	122.2	7.22	1.77	1.70	0.0789a	5.54
Hawassa	21.23a	129.2	7.81	1.69	1.71	0.0782a	5.50
Ziway	12.17b	115.8	7.39	1.65	1.74	0.0647b	5.47

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P \leq 0.05$.
Absence of letter in a grouping column denotes non significance
% determined on the basis of g 100 g^{-1} of pod dry weight
†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)
‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)
§ Data only 2012

Table 4.2. Nitrogen treatment by cultivar interaction for snap bean pod texture and pod appearance. Nitrogen treatment by location interaction for snap bean pod texture. Location by cultivar interaction for total soluble solids (TSS). (2011 and 2012)

	Nitrogen treatment			**Nitrogen treatment**			**Location**		
	Texture (1-5)†			Appearance (1-5)‡			TSS (^{o}Brix)§		
	0 kg N ha^{-1}	Rhizobium etli (HB 429)	100 kg N ha^{-1}	0 kg N ha^{-1}	*Rhizobium Etli* (HB 429)	100 kg N ha^{-1}	Debre Zeit	Hawassa	Ziway
Cultivar									
Andante	1.61cde	1.67de	1.00e	1.83bcd	1.78bcd	1.11e	5.6ab	5.57a-d	5.16f
Boston	1.61cde	1.67de	1.06e	1.83bcd	1.78bcd	1.00e	5.6ab	5.2ef	5.42bcd
Contender	1.83bcd	1.83bcd	1.00e	1.94bcd	1.67cd	1.06e			
Blue							5.52a-d	5.53a-d	5.35de
Lomami	1.83bcd	1.94abcd	1.00e	1.94bcd	1.89bcd	1.06e	5.42bcd	5.5a-d	5.61ab
Melkassa 1	2.61a	2.33abc	1.83bcd	2.83a	2.06b	1.89bcd	5.51a-d	5.38cde	5.59abc
Melkassa 3	2.5ab	2.17abcd	1.78bcd	2.78a	2.11b	1.56cd	5.53a-d	5.6ab	5.53a-d
Paulista	1.89bcd	1.83bcd	1.00e	1.94bcd	1.56d	1.00e	5.57a-d	5.62ab	5.53a-d
Volta	2bcd	1.83bcd	1.00e	1.94bcd	1.67cd	1.00e	5.54a-d	5.58abc	5.57a-d
Location									
Debre Zeit	2.15a	1.92abc	1.25bc						
Hawassa	1.85abc	1.85abc	1.21bc						
Ziway	1.81abc	1.96abc	1.17c						

Means followed by different letters in the same interaction groups (nitrogen treatment x cultivar; nitrogen treatment x location; location x cultivar) in the same parameter differ significantly based on LSD, $P \leq 0.05$

†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)

‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)

§ data only 2012

4.4.2 Nutrient concentrations

Analysis from combined data showed that N treatment, cultivar and location had no significant effect on the protein concentrations of snap bean pods. However, year by cultivar by location interaction was significant. This indicates that year had significant influence on cultivar by location interaction. The separate analysis for each year, (2011 [*P*=0.0186] and 2012 [*P*=0.0001]) showed that there were significant interactions for cultivar by location interactions. Paulista at Debre Zeit and Volta at Hawassa produced numerically the highest protein in 2011 and 2012, respectively (Table 4.4). Melkassa 3 at Debre Zeit in 2011 and the same cultivar at Ziway in 2012 produced numerically the lowest protein (Table 4.4). Other cultivars were inconsistent from location to location and year to year. Generally, most cultivars produced high protein at Ziway in 2011, and at Hawassa in 2012 (Table 4.4).

The combined data from the two years (2011 and 2012) showed that N treatment, cultivar, location and N treatment by location interaction all had significant effects on the protein content of snap bean pods. Moreover, the year by cultivar by location interaction was significant (Appendix 6). This shows that year had a significant influence on the cultivar by location interaction. The separate analysis for each year, 2011 (P = 0.0179) and 2012 (P = 0.0078), indicated that there were significant interactions for cultivar by location.

Applied N at Hawassa resulted in the greatest protein content but numerically the lowest was from the control treatment at Ziway (Table 4.5). The control treatment at Debre Zeit and Hawassa produced similar protein content to the applied N treatment at Ziway (Table 4.5). Generally protein content was higher at Hawassa followed by Debre Zeit (Table 4.5).

For the cultivar by location interaction, Boston at Hawassa had numerically the highest protein content in 2011 (Table 4.6). In 2012, Volta at the same site had numerically the highest protein and this cultivar had also in the top group in 2011 (Table 4.6). Andante in both years had numerically the lowest protein content (Table 4.6). Generally, cultivars had greater protein content at Hawassa followed by at Debrezeit (Table 4.6). Cultivars had the least protein at Ziway particularly in 2012 (Table 4.6).

The effects of N treatment and cultivar were significant on the P concentration of snap bean pods. Location had no effect on P concentrations of pods. Applied N improved P

concentrations, but improvement due to rhizobium inoculation was not statistically different from the Zero N control (Table 4.3). Lomami produced the highest P concentrations, significantly more than Boston, Contender Blue, Melkassa 1 and Melkassa 3 and Volta. Volta was numerically the lowest of all and significantly lower than Andante and Lomami in P concentration (Table 4.3).

Snap bean cultivar and location both significantly affected Zn concentration in the pods. The N treatment did not significantly change Zn concentration in snap bean pods. Zinc concentrations from 100kg N ha^{-1} and rhizobium inoculation treated snap bean pods were numerically better than the control, although not statistically different. Numerically the highest Zn concentration was recorded from Andante. Zinc concentration among Andante, Boston, Contender Blue and Paulista pods was similar (Table 4.3). Zinc concentration was numerically the lowest in Volta and Melkassa 3 and these two cultivars were similar to Lomami, Melkassa 1 and Paulista (Table 4.3). Snap bean pods produced the highest Zn concentrations when grown at Hawassa followed by Debre Zeit (Table 4.3), whereas the lowest Zn concentration was at Ziway (Table 4.3).

The combined data analysis of the two years showed that cultivar had a significant effect on Ca concentrations in snap bean pods. However, Ca concentration was not affected by N treatment and location. The year by cultivar by location interaction was also significant (Appendix 6). The separate analysis for 2012 indicated that the cultivar by location (P = 0.0008) interaction significantly affected Ca concentration of snap bean pods. In 2011, the cultivar by location (P = 0.375) interaction was not significant. In the two years of combined data, Andante produced higher Ca concentration than Contender Blue and Melkassa cultivars (Table 4.3).The cultivar by location interaction result in 2012 showed that Andante produced the highest Ca when grown at Ziway (Table 4.4). Again Melkassa 3 had numerically the lowest Ca concentration at Ziway (Table 4.4). Cultivars had similar Ca concentrations at Debre Zeit. The same was true at Hawassa except for pods from Melkassa 1 (Table 4.4).

Potassium concentration in pods was not affected by N treatment, cultivar or location. But the cultivar by location interaction significantly affected K concentration of snap bean pods. Numerically, Lomami pods at Ziway had the highest K concentration but Melkassa 1 pods at Debre Zeit had the least (Table 4.5). Overall, Lomami was also the most consistent cultivar in K

concentration across all locations found (Table 4.5). Melkassa cultivars had lower K at Hawassa than other cultivars in the same location (Table 4.5).

The interaction of N treatment by location significantly affected K concentration of snap bean pods. Applied N at Ziway resulted in higher K concentration than rhizobium inoculation and Zero N at Debre Zeit (Table 4.5). Generally, snap bean cultivars produced pods with numerically lower K concentration at Debre Zeit than Hawassa and Ziway (Table 4.5).

Table 4.3. Protein, phosphorus, zinc, calcium and potassium concentrations of snap bean pods affected by nitrogen treatment, cultivar and location (2011 and 2012)

	Protein concentration (%)	Phosphorus (%)	Zinc (ppm)	Calcium (%)	Potassium (%)
Nitrogen treatment					
0 kg N ha^{-1}	17.9	0.399b	28.96	0.68	3.2
Rhizobium etli (HB 429)	18.1	0.406ab	29.42	0.67	3.1
100 kg N ha^{-1}	18.4	0.413a	29.93	0.69	3.2
Cultivar					
Andante	18.6	0.413ab	31.21a	0.76a	3.2
Boston	18.1	0.401bc	30.70ab	0.70ab	3.2
Contender Blue	18.3	0.406bc	30.88a	0.65bc	3.3
Lomami	18.9	0.423a	29.69abc	0.69ab	3.3
Melkassa 1	17.7	0.397bc	28.36bc	0.64bc	3.0
Melkassa 3	16.9	0.400bc	28.13c	0.59c	3.1
Paulista	18.0	0.411abc	29.06abc	0.72ab	3.3
Volta	18.2	0.394c	27.45c	0.68ab	3.2
Location					
Debre Zeit	18.3	0.404	27.87b	0.66	2.9
Hawassa	18.3	0.415	36.22a	0.61	3.2
Ziway	17.7	0.399	24.20c	0.77	3.4

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P \leq 0.05$. Absence of letter in a grouping column denotes non significance

% determined on the basis of g 100 g^{-1} of pod dry weight.

ppm=µg g^{-1}

Table 4.4. Protein (%) and calcium (%) concentrations of snap bean pods affected by cultivar by location interaction in 2011 and 2012

	2011			2012			2012		
	Location			**Location**			**Location**		
	Protein concentration (%)			Protein concentration (%)			Calcium (%)		
Cultivar	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	17.54a-g	16.19g-j	18.16a-e	19.93a-f	20.49abc	19.26b-g	0.757b-e	0.632f-k	1.016a
Boston	17.31a-g	16.32f-i	17.13b-h	18.83d-j	20.84a	17.91h-l	0.763b-e	0.616g-k	0.793bc
Contender Blue	17.69a-g	15.18ij	18.90abc	19.81a-g	20.80a	17.41j-m	0.667d-i	0.550jl	0.769bcd
Lomami	18.34a-d	17.05d-h	18.42a-d	20.12ab	21.07a	18.48f-i	0.658d-j	0.551ijl	0.819b
Melkassa 1	16.32f-i	14.88ij	17.19b-h	19.69a-g	20.87a	17.13lm	0.743b-f	0.659d-g	0.697e-f
Melkassa 3	14.38j	15.45hij	18.05a-f	18.97c-h	19.74b-g	15.03n	0.691c-h	0.550jl	0.530kl
Paulista	18.91ab	16.37e-i	17.42a-g	18.87d-j	20.12a-e	16.47m	0.713b-g	0.615g-k	0.804bc
Volta	17.21c-h	15.91g-j	18.17a-e	18.67e-k	21.10a	18.32g-k	0.664d-j	0.562ijl	0.793bc

Means followed by the different letters in the same interaction group (cultivar x location) differ significantly based on LSD, $P \leq 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g

% determined on the basis of g 100 g^{-1} of pod dry weight

Table 4.5. Nitrogen treatment by location interaction for snap bean pod protein content (kg ha^{-1}) in 2011 and 2012

	Combined (2011 and 2012)		
	Location		
	Protein content (kg ha^{-1})		
Nitrogen treatment	Debre Zeit	Hawassa	Ziway
0 kg N ha^{-1}	389de	375ef	236g
Rhizobium etli (HB 429)	479cd	545b	281fg
100 kg N ha^{-1}	514bc	668a	340ef

Means followed by the different letters in the same interaction group (nitrogen treatment x location) differ significantly based on LSD, $P \leq 0.05$

Table 4.6. Protein (kg ha^{-1}) content of snap bean pods affected by the cultivar by location interaction in 2011 and 2012

	2011			**2012**		
	Location			**Location**		
	Protein content (kg ha^{-1})			Protein content (kg ha^{-1})		
Cultivar	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	290jkl	338g-k	222l	391gh	407fgh	138j
Boston	472a-e	535a	258kl	502c-f	645ab	214ij
Contender Blue	499ab	424b-h	395d-i	453e-h	545cde	207ij
Lomami	491a-d	475a-e	436a-g	530b-e	633b	263i
Melkassa 1	400c-i	511ab	354g-j	577bc	612bc	350h
Melkassa 3	412b-h	464a-f	341h-k	467d-g	578bcd	242i
Paulista	396e-h	501abc	326h-k	505c-f	555b-e	255i
Volta	467a-f	532a	368f-j	517c-f	743a	203ij

Means followed by the different letters in the same interaction group (cultivar x location) differ significantly based on LSD, $P \leq 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g

Table 4.7. Potassium (%) of snap bean pods as affected by the cultivar by location interaction in 2011 and 2012

	Combined (2011 and 2012)		
	Location		
	Potassium (%)		
	Debre Zeit	Hawassa	Ziway
Nitrogen treatment			
0 kg N ha^{-1}	2.83c	3.29abc	3.39ab
Rhizobium etli (HB 429)	2.87bc	3.24abc	3.28abc
100 kg N ha^{-1}	2.98abc	3.18abc	3.47a
Cultivar			
Andante	2.86f-j	3.40a-d	3.20d-h
Boston	2.78g-j	3.26a-g	3.42a-e
Contender Blue	3.02b-g	3.38a-f	3.50abc
Lomami	3.08a-f	3.25a-g	3.58a
Melkassa 1	2.63j	2.95e-j	3.36a-f
Melkassa 3	2.90d-h	3.02c-i	3.32b-f
Paulista	3.02b-g	3.33a-f	3.38a-f
Volta	2.87f-j	3.32a-f	3.25b-g

Means followed by different letters in the same interaction group (nitrogen treatment x location; cultivar x location) differ significantly based on LSD, $P \leq 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g

% determined on the basis of g 100 g^{-1} of pod dry weight

4.5 Discussion

Applied N and rhizobium inoculation were effective in improving the marketable yield of snap bean pods by 43 and 18 %, respectively (Table 4.1). Our results agreed with El-Awadi et al. (2011), Salinas-Ramírez et al. (2011) and Mahmoud et al. (2010), all of whom reported that applied N improved yield and yield components of common bean. Results also confirmed Bildirici and Yilmaz (2005) who reported yield improvement by rhizobium inoculation for common bean. Our improved yield response with rhizobia inoculation was in contrast to Otieno et al. (2009), who found no yield response to rhizobium inoculation in common bean. However, these reports were focused on grain yield. Our results further demonstrated that the benefit of rhizobium inoculation can be realized in improving the quality of snap bean pods harvested at immature stages. Our investigation showed the possibility of producing export quality snap bean under reduced inputs that minimizes the reliance of vegetable production on heavy N fertilizer rates, especially for resource limited farmers. The marketable yield obtained in the current experiment under rain fed conditions was comparable with the yield reported by Salinas-Ramírez et al. (2011) from Mexico (18.8 t ha^{-1}), El-Yazied et al. (2012) from Egypt (18.7 t ha^{-1}), and Salem and Midan (2012) from Egypt (21.0 t ha^{-1}).

The distinct differences among cultivars for marketable yield may be due to the size of the plant that contributed to increased photosynthetic area (leaf area index). Melkassa 1, the top cultivar in marketable yield, was characterized by tall plants and a larger leaf area index (Appendix 9) that determined its' high yield capacity. In addition, Melkassa 1 was a well-adapted cultivar to a reduced input production system, especially dry land agriculture, and it also had better performance because this cultivar was developed in Ethiopian conditions. The yield potential of commercial cultivars may be limited by environmental variables, potential moisture, as this experiment was conducted under natural rain fed conditions. The marketable yield of snap bean cultivars was lower at Ziway (Table 4.1) but yield from Debre Zeit and Hawassa were similar. Ziway is characterized by a high pH soil, semi-arid environment with erratic and unpredictable rain fall (Appendices 1 and 2). This may limit the productivity and quality of snap bean. The high marketable yield at Debre Zeit and Hawassa may be due to suitability of the agro-ecology at these locations for enabling better utilization of soil fertility (Appendices 1 and 2).

Length and diameter of snap bean pods were not affected by N treatment and location under rain fed conditions (Table 4.1). Pod size is therefore highly controlled by genetic factors (additive gene effect) and less affected by environmental factors (Arunga et al., 2010). From our study, cultivars could be grouped into three categories based on pod diameter. Andante is an extra fine cultivar with very small pod diameter ranging from 5.0 mm to 6.2 mm and Melkassa cultivars (Melkassa 1 and Melkassa 3) were at the other extreme, being bobby pod type cultivars with pod diameters ranging from 8.0 mm to 8.7 mm (Tables 4.1). The remaining cultivars were fine cultivars with pod diameter of 7.0 mm to 7.6 mm (Wahome et al., 2013).

Texture and appearance of snap bean pods are the two most decisive parameters that influence marketability. Snap bean pods are graded into marketable and unmarketable pods depending on texture and appearance. Texture and appearance of pods again depend on smoothness, uniformity and overall look of the pods in the absence of disease, insect damage and other defects. The appearance of pods was improved by application of N fertilizer (Table 4.1 and 4.2). The texture and appearance of all the commercial cultivars were improved by N fertilizer application. Melkassa cultivars responded well for rhizobium inoculations especially pod appearance (Table 4.2). The result is in agreement with previous studies stating N application increased the quality of green bean (Mahmoud et al., 2010; Kamanu et al., 2012). Our findings demonstrated that rhizobium inoculant can provide sufficient N to improve the appearance of snap bean pods at least for some cultivars. Improved N nutrition turned green pods into ones that were well-formed and straight, bright in color and of acceptable quality.

Commercial cultivars produced the highest quality pods due to their fine texture, and well-rounded straight pods. Melkassa cultivars lacked some quality characteristics including smoothness and uniformity of pods, particularly for Melkassa 1 which had a high marketable yield. Therefore, breeding work is needed to improve the pod appearance for Melkassa 1 to bring this cultivar up to the premium level. Generally, all cultivars had fine texture and acceptable appearance scores of less than 3 at all locations. This indicates that it is possible to produce snap bean with acceptable texture and appearance for export markets even without N application and inoculation at any of the three sites.

Nitrogen application and rhizobium inoculation increased the TA of snap bean pods. Studies on tomato (Wright and Harris, 1985; Erdal et al., 2007) and grape (Baiano et al., 2011) fruits indicated that increasing N fertilizer increased TA of the fruit. As TA is the prime taste

quality determinant in fruit juice (Zagory and Kader, 1989), we assumed applied N and rhizobium inoculation would improve the taste quality of pods by increasing TA. The TA of the cultivars was in the range for snap bean determined by Proulx et al. (2010). The higher TA at Debre Zeit and Hawassa may be due to favorable growing conditions for snap bean production as reflected in other parameters such as marketable yield. Nitrogen nutrition, cultivar and growing location may additionally affect the taste quality of snap bean in terms of TA.

Some cultivars had consistent pod TSS from location to location but others did not. Melkassa 3, Paulista and Volta were numerically the most stable cultivars found in the top group of pod TSS at all locations. This may be due to environmental variables of a specific location determining the TSS of a particular cultivar (Hoogenboom, 2000). In soybean, TSS of pods is directly associated with the photo-assimilate manufactured by the plant (Liu et al., 2011), and affects the relative concentrations of soluble sugars in the pod. Generally, factors that affect soluble sugars also influence TSS (Caliman et al., 2010). TSS is another taste quality determinant (Champa et al., 2008), and cultivars with higher TSS have higher taste quality particularly when in combination with high TA (Al-Jamali and Hani, 2009).

In 2012, a cultivar by location interaction resulted in generally higher pod protein concentration at Hawassa but the reverse was observed in 2011 with numerically better protein at Ziway. The low pod protein concentrations for most of the cultivars at Ziway may be due to unfavorable weather conditions especially erratic and excess rain fall during the early growth periods of these snap bean in 2012. Nitrogen from agricultural fields may be lost by moderate to high rain fall (Scharf and Lory, 2006), an effect which would be magnified by the sandy nature of the soil at Ziway. Pod protein concentrations of cultivars were inconsistent from year to year and location to location. Snap bean pods are rich in protein. The protein concentration ranged from 16 to 21% on dry weight basis or 1.3 to 1.8% on fresh weight basis under rain fed conditions.

The performance cultivars for protein content were the result of protein concentrations and amount of yield. Unlike protein concentration, protein content was significantly affected by N treatment by location interaction. It is clear that the yield influence of N treatment play a major role for protein content. The protein content of snap bean had a mostly similar pattern with the yield. Cultivars had numerically better protein concentration at Ziway in 2011 than other locations. However, the pattern was different for protein content.

Applied N increased the P concentration in snap bean pods. This result was supported by Apthorp et al. (1987) who reported that N fertilizer application increased P uptake by plants. Rhizobium inoculation and applied N were similar in increasing pod P concentration. However, only the latter was significantly different from the control. The concentrations of P in green pods of snap bean showed variation among cultivars. Phosphorus shoot tissue concentration and its uptake by the plant were affected by varietal differences in common bean (Mourice and Tryphone, 2012).

Applied N and rhizobium showed a trend of enhanced Zn pod concentrations of snap bean pods numerically (Table 4.3). The variations among cultivars for Zn are supported by the report from Beebe et al. (2000) and Gregorio (2002) who reported sufficient variability in Zn concentration among bean cultivars. We found that our cultivars had pod Zn concentrations close to mean values reported by Beebe et al. (2000). Zinc concentration of pods was highest at Hawassa followed by Debre Zeit. The soil analysis from each locations showed that high Zn content in the soil was found at Hawassa followed by Ziway, and Debre Zeit had the least. This may suggest that a high Zn concentration in pods at Hawassa was due to high Zn content in the soil. However, the pod Zn concentration at Debre Zeit was higher than at Ziway. This may indicate that environmental variables other than Zn concentration in the soil may also contribute to Zn concentration in snap bean pods. Studies indicated that higher pH reduced the availability and plant uptake of Zn in the soil solution (Jeffery and Uren, 1983), which may explain the lower Zn concentration in pods at Ziway where high soil pH occurred (Appendix 2).

The result from 2012 showed that Andante was the top cultivar in accumulating Ca in its pod when grown at Ziway. This result is in agreement with reports of cultivars with small pod diameters having a greater Ca concentration (Grusak and Pomper, 1999). Generally, snap bean cultivars had numerically, greater pod Ca when grown at Ziway and Debre Zeit. Low Ca concentration in pods at Hawassa may be due to lower Ca concentration in the soil. Calcium concentration in snap bean pods is influenced by cultivar and environmental conditions such as heat units (temperature), rainfall and water availability for crop uptake, and soil Ca concentration (Quintana et al., 1999b).

Applied N at Ziway improved the K concentration of snap bean pods when compared to rhizobium inoculation and Zero N at Debre Zeit. Hirzel and Walter (2008) also reported that NPK fertilizer application increased soil and tissue concentrations of K in sweet corn.

4.6 Conclusions

Nitrogen application and rhizobium inoculation increased marketable yield and TA of snap bean compared to a Zero N control. Nitrogen treatments interacted with cultivars to affect pod texture and pod appearance. Nitrogen application was almost always better thanrhizobium inoculation for improving pod appearance, and consistently resulted in improved pod appearance compared to a Zero N control. However, rhizobium inoculation also imp roved the appearance, particularly of the two Melkassa cultivars. Melkassa 1 was well-adapted to rain-fed conditions in that it gave numerically the highest overall marketable yield across all locations. Melkassa 1 had the largest pod diameter of any tested cultivar, and it frequently ranked below commercial cultivars for pod texture and pod appearance. Locations interacted with cultivars and affected the pod traits TSS and concentrations of protein, Ca, and K. Snap bean pods produced at Debre Zeit and Hawassa were similar in marketable yield and several other traits. Pod Zn concentration was particularly high at Hawassa. Ziway, with a more arid climate and soil pH above 8.0, was the least favorable location for production of export-quality snap bean compared to the other two locations tested. Generally, production of marketable quality snap bean pods can be achieved by using rhizobial inoculation as N source particularly for resource limited farmers.

4.7 Prologue to chapter 5

In chapter 4, we found that rhizobial inoculation increased marketable yield and TA of snap bean pods as compared to the uninoculated and unfertilized treatment. Additionally, N treatment interacted with cultivars to affect pod texture and appearance of snap bean. However, the effects of N treatment (applied N and rhizobial inoculation), cultivar difference and agro-ecological (location) variations on snap bean marketable pod yield, other physical qualities such as texure appearance, TSS, TA, and pod nutrient concentrations have not been researched under irrigated conditions. Snap bean production in eastern Africa particularly in Ethiopia is majorly under irrigation during the dry season. Therefore, a study in chaper 5 was conducted to investigate the influence of N treatment, cultivar and agro-ecological differences on marketable pod yield, quality and nutrient concentrations of snap bean under irrigated conditions.

CHAPTER 5

5. Pod Quality of Snap Bean Cultivars in Response to Nitrogen Treatment and Agro-ecology under Irrigation

5.1 Abstract

Snap bean (*Phaseolus vulgaris* L.) is one of the major vegetable crops in Ethiopia grown for export market. The crop is mainly produced during the dry season under irrigation. Local farmers are producing snap bean because it is more profitable than other vegetables for local markets. The productivity and quality of snap bean depend on crop nutrition, cultivars and growing environment. The objective of this research was to investigate the influence of N treatment, cultivar and contrasting environment differences (locations) on pod quality of snap bean under irrigation. Three N treatments (0 and 100 kg N ha^{-1}, and *Rhizobium etli* [HB 429]) and eight snap bean cultivars were factorially arranged as a randomized block design with three replications under irrigation in 2012 at three locations (Debre Zeit, Hawassa and Ziway). Marketable yield, texture, appearance of pods; and nutrient concentrations such as protein, Zn, iron, Ca and K concentrations were recorded. Applied N increased marketable yield by 33% but rhizobium inoculation was not significantly different from the untreated control. Melkassa 3 was the better cultivar producing higher protein, P and K concentrations, particularly at Hawassa. Generally, Hawassa was the site where greatest marketable yield, protein, K and Zn concentrations found in snap bean pods.

Key words: Nitrogen, Rhizobium, quality; snap bean

5.2 Introduction

Snap bean (*Phaseolus vulgaris* L.) is an important vegetable legume crop grown in Ethiopia for export and local markets. Economically the price of snap bean is by far better than other vegetable crops in local markets. This crop has been grown in many parts of Ethiopia mainly in the central Rift Valley. Snap bean is mainly grown under irrigation during the dry season for export. Snap bean needs a continuous supply of water, particularly during pod set and pod growth stages (Strang, 2011). Pods of premium quality can be produced under proper irrigation (Sezen et al., 2005). Snap bean yield and quality under irrigation production system is

assumed to be high because water shortage is minimized (Sezen et al., 2008). Appropriate N fertilizer improves yield and quality of snap bean (Hochmuth, 1997; Hochmuth and Hanlon, 2010). Reports indicated that increasing NPK fertilizer rate increased yield and protein content of snap bean pods but had no effect on pod length and diameter (Abdel-Mawgoud et al., 2005).

The use of rhizobia inoculation as source of N is effective in many legume field crops (Bhuiyan et al., 2008; Sall and Sinclair, 1991; Bildirici and Yilmaz, 2005; Otieno et al., 2009). Successful nodulation and functionality of nodules in N_2 fixation depends on many factors including growing conditions, legume-rhizobia combination (Zahran, 1999) and nitrate-limiting conditions (Schumpp et al., 2009). The productivity and quality of snap bean also depends nutrition, cultivars and agro-ecological conditions. Agro-ecological conditions as a cumulative effect of climate and edaphic factors significantly effects yield, nutritional and other qualities of bean (Kigel, 1999). In the previou chapter (chapter 4), we dealt with similar type of study but under rain fed conditions. Therefore, this experiment was designed to investigate the influence of N treatment, cultivar and contrasting environment (location) differences on pod quality of snap bean under irrigated conditions.

5.3 Materials and Methods

5.3.1 Experimental Sites

The study was conducted under irrigation at Debre Zeit, Hawassa and Ziway, with details in Chapter 3.3.1 and Appendices 1 and 2.

5.3.2 Experimental design and crop management

The experiment was seeded on February 8, 9, and 12 at Ziway, Debre Zeit and Hawassa, respectively. Furrow irrigation was applied starting from the date of seeding to the end of harvesting at four-day intervals based on evaporation demand and local experience for snap bean production. Plots were furrow irrigated until the soil ridges were saturated. Three N treatments were tested against eight snap bean cultivars factorially arranged in a completely randomized block design with three replications. The detailed experimental design and crop management was documented in Chapter 3.3.2.

5.3.3 Measurements

Data on marketable yield, pod length, pod diameter, total soluble solids (TSS), titratable acidity (TA), protein, phosphorus (P), zinc (Zn), iron (Fe), calcium (Ca) and potassium (K) concentrations were determined according to procedures listed in Chapter 4.3.3.

5.3.4 Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS software version 9.3 (SAS Institute Inc., 2012) to determine Analysis of Variance (ANOVA). Assumptions of ANOVA were checked for normality of distribution and homogeneity of variance. Nitrogen treatment, cultivar and location (agro-ecology) were designated as fixed effects and replication (block) as a random. Locations represented three distict agro-ecologies (climate zones). The DDFM=Kr option was used for approximating the degrees of freedom for means. Treatments were compared by the LSD method at $P \leq 0.05$. The *P*-values from mixed model ANOVA F-test Tables for the response variables are presented on Appendices 11 and 12.

5.4 Results

5.4.1 Pod marketable yield and other physical qualities

Nitrogen treatment, cultivar, location and cultivar by location interaction significantly affected marketable yield and pod length under irrigated conditions. The N by location interaction also affected pod length. Pod diameter was significantly affected by cultivar and the cultivar by location interaction. Pod curving was not affected by any of the factors. Texture and appearance of pods were significantly affected by N treatment, cultivar, cultivar by location, N treatment by cultivar and N treatment by location interactions. Titratable acidity and TSS were significantly affected by the N treatment and cultivar by location interaction. Total soluble solids were also affected by the N treatment by location interaction.

Applied N at the rate of 100 kg N ha^{-1} increased marketable yield and pod length of snap bean cultivars as compared to rhizobium inoculation and the control (0 kg N ha^{-1}) (Table 5.1). There was no significant difference between the latter two.

Table 5.1. Pod marketable yield, length and diameter of snap bean cultivars in 2012 under irrigation

Nitrogen treatment	Marketable Yield (t ha^{-1})	Pod length (mm)	Pod diameter (mm)
0 kg N ha^{-1}	22.1b	118.9b	7.40
Rhizobium etli (HB 429)	23.3b	120.5b	7.40
100 kg N ha^{-1}	29.4a	122.3a	7.40

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P<0.05$. Absence of letter in a grouping column denotes non significance

The highest marketable yield was produced by Contender Blue at Hawassa but it was similar to Paulista and Volta at the same location (Table 5.2). Numerically the lowest marketable yield was produced by Andante at Ziway (Table 5.2). Generally all cultivars produced higher marketable yield at Hawassa than other locations (Table 5.2). Numerically Boston was better at Debre Zeit, Contender Blue at Hawassa and Melkassa 1 at Ziway in marketable yield (Table 5.2). Melkassa 3 produced the longest pods at Debre Zeit, but Andante produced the shortest pod particularly at Ziway (Table 5.2). Numerically the largest pod diameter was produced by Melkassa 1 at Debre Zeit. Both Melkassa cultivars had the same pod diameter at Debre Zeit (Table 5.2). The pod diameter of Melkassa 1 was not affected by location.

Cultivars produced pods with the finest texture under applied N (Table 5.3). However, the pod texture of Andante, Boston, Contender Blue and Volta was not affected by any N treatment because it was already optimal without treatment (Table 5.3). The pod texture of Lomami and Paulista was improved by only applied N (Table 5.3) but the pod texture of both Melkassa cultivars was improved by both applied N and rhizobium inoculation (Table 5.3). For the N treatment by location interaction, the applied N at Debre Zeit and Ziway and the rhizobium inoculation at Debre Zeit improved the texture of snap bean pods within each location (Table 5.4). The texture of snap bean pods at Hawassa was already optimal without N treatment. The texture of pods of most of the commercial cultivars was finest at all locations but the texture of Melkassa cultivars was coarser at Ziway compared to the other locations (Table 5.4).

Applied N within each location improved the appearance of pods of snap bean cultivars except Andante and Boston which was already optimal (Table 5.3). The appearance of Contender Blue, Lomami, Melkassa 1, Paulista and Volta were improved by rhizobium inoculation (Table 5.3).

Table 5.2. Pod marketable yield, length and diameter of snap bean as influenced by cultivar by location interaction in 2012 under irrigation

Cultivar	Location			Location			Location		
	Marketable yield (t ha^{-1})			Pod length (mm)			Pod diameter (mm)		
	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	18.2jk	24.7d-g	14. 8k	109ij	108ij	106j	5.46j	6.13i	6.12i
Boston	26.0def	28.6cd	21.3f-j	125b-f	121efg	127b-e	7.20fgh	7.16fgh	7.18fgh
Contender Blue	23.1e-i	36.2a	19.5h-k	117gh	113hi	113hi	98gh	7.35e-h	7.54def
Lomami	23.7de-i	31.3bc	18.8ijk	125b-f	116gh	122efg	7.20fgh	6.84h	7.62def
Melkassa 1	22.8e-j	27.0cde	25. 6d-g	124c-f	123def	113hi	8.65a	8.48ab	8.39ab
Melkassa 3	20.5g-j	28.5cd	23.2e-j	137a	129bcd	130bc	8.36abc	7.56def	8.09bcd
Paulista	23.2e-i	36.2a	22.9e-j	122efg	121efg	122efg	7.29e-h	7.58def	7.52efg
Volta	25.2def	33.5ab	23.6de-h	130b	120fg	124def	7.82cde	7.16fgh	7.67def

Means followed by different letters in the same interaction group (cultivar x location) in the same parameter differ significantly based on LSD, $P < 0.05$. Letters d-g indicate all alphabetical letters included in the range from d to g

Table 5.3. Pod texture and appearance of snap bean as influenced by cultivar by nitrogen treatment interaction in 2012

	Nitrogen treatment			Nitrogen Treatment		
	Texture (1-5)†			Appearance (1-5)‡		
Cultivar	0 kg N ha^{-1}	*Rhizobium etli* (HB 429)	100 kg N ha^{-1}	0 kg N ha^{-1}	*Rhizobium etli* (HB 429)	100 kg N ha^{-1}
Andante	1.00h	1.11gh	1.00h	1.22ijk	1.33hij	1.00k
Boston	1.00h	1.02h	1.00h	1.22ijk	1.11jk	1.00k
contender Blue	1.22fgh	1.11gh	1.00h	1.67fg	1.22ijk	1.11jk
Lomami	1.56e	1.33efg	1.00h	1.78ef	1.44ghi	1.00k
Melkassa 1	2.67a	2.22bc	1.89d	3.00a	2.56b	2.22cd
Melkassa 3	2.44ab	2.11cd	1.89d	2.67b	2.44bc	2.00de
Paulista	1.44ef	1.33efg	1.00h	2.11d	1.67fg	1.00k
Volta	1.00h	1.00h	1.00h	1.56fgh	1.22ijk	1.00k

Means followed by different letters in the same interaction group (nitrogen treatment x cultivar) in the same parameter differ significantly based on LSD, $P \leq 0.05$

†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)
‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)

For the N treatment by location interaction, applied N at Debre Zeit and Ziway and the rhizobium inoculation at Debre Zeit both improved the appearance of snap bean pods (Table 5.4). The appearance of pods at Hawassa was at best quality even without N application or rhizobium inoculation (Table 5.4). A cultivar by location interaction showed that most cultivars generally had the best pod appearance at Hawassa followed by Debre Zeit (Table 5.4). Melkassa 1 and Paulista produced superior pods at Debre Zeit compared to other two locations (Table 5.4).

Applied N at all locations and rhizobia inoculation at Debre Zeit reduced the TSS of snap bean pods (Table 5.5). The TSS of pods from Volta was numerically the highest at Debre Zeit but the least was from Boston at Ziway (Table 5.5). Numerically the highest TA was obtained from Andante at Hawassa but the least was from Melkassa 3 at Ziway (Table 5.5). Generally, cultivars produced better TA at Debre Zeit and Hawassa (Table 5.5).

Table 5.4. Pod texture and appearance of snap bean as influenced by nitrogen treatment by location, and cultivar by location interactions in 2012 under irrigation

	Location			**Location**		
	Texture (1-5) †			Appearance (1-5) ‡		
	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Nitrogen treatment						
0 kg N ha^{-1}	1.54ab	1.42abc	1.67a	2.04a	1.58b	2.08a
Rhizobium etli (HB 429)	1.21cd	1.34bcd	1.67a	1.38bc	1.50b	2.00a
100 kg N ha^{-1}	1.21cd	1.21cd	1.25bcd	1.29bc	1.29bc	1.29bc
Cultivar						
Boston	1.00g	1.02g	1.00g	1.11ij	1.00j	1.22hi
Contender Blue	1.00g	1.11efg	1.22efg	1.44fg	1.11ij	1.44fg
Lomami	1.22efg	1.22efg	1.44ef	1.33gh	1.33gh	1.56f
Melkassa 1	2.00cd	2.11bc	2.67a	2.33c	2.67ab	2.78a
Melkassa 3	2.22bc	1.78de	2.44ab	2.56b	1.89d	2.67ab
Paulista	1.11efg	1.33ef	1.33ef	1.33gh	1.67ef	1.78de
Volta	1.00g	1.00g	1.00g	1.33gh	1.00j	1.44fg

Means followed by different letters in the same interaction group (nitrogen treatment x location; cultivar x location) in the same parameter differ significantly based on LSD, $P \leq 0.05$

†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)
‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)

Table 5.5. Pod total soluble solids (TSS) and titratable acidity (TA) of snap bean as influenced cultivar by location, and nitrogen treatment by location interactions in 2012 under irrigation

	Location			**Location**		
	TSS (^{o}Brix)			TA (%)		
Cultivar	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	6.11a-d	6.11a-d	6.33abc	0.074abc	0.078a	0.057efgh
Boston	6.17a-d	6.33abc	5.44f	0.071a-e	0.063b-h	0.061c-h
Contender Blue	5.83def	6.06a-e	6.11a-d	0.069a-f	0.073abc	0.058e-h
Lomami	6.28a-d	5.61ef	6.5ab	0.075ab	0.059d-h	0.067a-f
Melkassa 1	6.17a-d	6.17a-d	6.28a-d	0.071a-e	0.068a-f	0.056fgh
Melkassa 3	6.17a-d	5.89c-f	6.00cde	0.053h	0.074abc	0.051h
Paulista	6.06b-e	6.06a-e	6.17a-d	0.067a-g	0.074abc	0.053gh
Volta	6.51a	5.94cde	6.22a-d	0.073a-d	0.069a-f	0.067a-f
Nitrogen treatment						
0 kg N ha^{-1}	6.50a	6.21abc	6.42ab			
Rhizobium etli (HB 429)	6.00c	6.17bc	6.48a			
100 kg N ha^{-1}	5.98cd	5.69de	5.50e			

Means followed by different letters in the same interaction group (cultivar x location; nitrogen treatment x location) in the same parameter differ significantly based on LSD, $P \leq 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g,
% determined on the basis of g 100 g^{-1} of pod dry weight

5.4.2 Nutrient concentrations

The cultivar, location, and cultivar by location interaction significantly affected protein, P, Ca and K concentrations under irrigation. Nitrogen treatment significantly affected protein concentration. Zinc concentration was significantly affected by location. Iron was affected by none of the factors.

Melkassa 3 produced the highest protein concentration at Hawassa except Melkassa 1 at the same location. The same cultivar (Mekassa 3) produced the least protein concentration at Debre Zeit but comparable with Andante and Melkassa 1 (Table 5.6). Generally, cultivars produced higher protein concentration at Hawassa followed by Ziway but protein concentration was lowest at Debre Zeit (Table 5.6).

Melkassa cultivars produced the highest P concentration at Hawassa which was similar to Lomami (Table 5.6). Numerically the least P was from Boston at Ziway followed by Volta (Table 5.6). Generally, cultivars produced higher P concentration at Hawassa followed by Debre Zeit, but lowest P concentration was in pods from Ziway (Table 5.6).

Andante produced the highest pod Ca concentration at Ziway, similar to Lomami, Paulista and Volta at the same location, and similar to Andante at Debre Zeit (Table 5.6). Numerically, the least pod Ca concentration was produced by Melkassa 1 at Debre Zeit (Table 5.6). Generally, Andante was cultivar with the best pod Ca concentration within each location, and Ca concentration was overall better at Ziway (Table 5.6).

The highest K concentration was produced by Contender Blue at Hawassa, comparable to Lomami, Melkassa cultivars and Paulista (Table 5.7). Most cultivars produced better K concentrations at Hawassa followed by Ziway (Table 5.7).

The highest snap bean pod Zn concentration was obtained at Hawassa (Table 5.7). But Zn concentrations at Debre Zeit and Ziway were comparable (Table 5.7).

Table 5.6. Protein (%), phosphorus (%) and calcium (%) concentrations of snap bean pods affected by cultivar by location interaction in 2012 under irrigation

	Location			Location			Location		
	Protein (%)			Phosphorus (%)			Calcium (%)		
Cultivar	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	16.71jk	18.65e-h	18.03f-j	0.407d-i	0.368hi	0.381f-i	0.795a-d	0.727c-f	0.849a
Boston	18.37efg	22.30bc	18.54e-h	0.439c-f	0.469c	0.354i	0.729cde	0.668e-h	0.804abc
Contender Blue	17.44g-j	21.96bc	19.14de	0.423c-h	0.475bc	0.392e-i	0.664e-h	0.665e-h	0.744b-e
Lomami	18.06e-h	23.02ab	19.06def	0.432c-g	0.526ab	0.402e-i	0.647fgh	0.605h	0.797a-d
Melkassa 1	16.96ijk	22.22bc	19.03def	0.387f-i	0.541a	0.390e-i	0.610h	0.710efg	0.701efg
Melkassa 3	15.93k	23.66a	18.62e-h	0.399e-i	0.536a	0.389e-i	0.637gh	0.682e-h	0.725def
Paulista	17.48g-j	20.46d	18.26e-i	0.474bc	0.463cd	0.375ghi	0.696efg	0.707efg	0.832a
Volta	17.13hij	21.71c	18.23e-j	0.446cde	0.462cd	0.3576i	0.695efg	0.680e-h	0.815ab

Means followed by different letters in the same interaction group (cultivar x location) in the same parameter differ significantly based on LSD, $P \leq 0.05$ Letters g-j indicate all alphabetical letters included in the range from g to j

% determined on the basis of g 100 g-1 of pod dry weight

Table 5.7. Potassium (%) concentration of snap bean as influenced by cultivar by location interaction and Zinc (ppm) at the three locations in 2012 under irrigation

	Potassium (%)		
Cultivar	Debre Zeit	Hawassa	Ziway
Andante	3.23ghi	3.62ef	3.52efg
Boston	3.53efg	4.14bc	3.35f-i
Contender Blue	3.48efg	4.58a	3.54efg
Lomami	3.45efg	4.46ab	3.73de
Melkassa 1	3.09hi	4.43ab	3.40e-h
Melkassa 3	3.01i	4.51a	3.53efg
Paulista	3.38e-h	4.31abc	3.47efg
Volta	3.25f-i	4.06cd	3.37f-i
	Zinc (ppm)		
Locations	Debre Zeit	Hawassa	Ziway
	27.9b	42.7a	26.7b

Means followed by different letters in cultivar x location differ significantly based on LSD, $P \leq 0.05$.
Means of zinc followed by different letters at different locations differ significantly based on LSD, $P \leq 0.05$

ppm=µg g^{-1} of pod dry weight
% determined on the basis of g 100 g^{-1} of pod dry weight

5.5 Discussion

These results demonstrated that applied N increased marketable yield by 33% under irrigated conditions. Pod length was also increased by applied N. Applied N is most effective in increasing productivity in seasons of sufficient soil moisture (Simonne et al., 2012). An adequate amount of irrigation water to maintain optimal soil moisture may have contributed to increased yield from the applied N treatment in the current experiment. Rhizobium inoculation had no significant improvement on marketable yield, length and diameter of snap bean pods. As reported by Zahran (1999) and Schumpp et al. (2009), effectiveness of rhizobium inoculant for N_2 fixation is affected by many factors including environmental conditions and rhizobium-host combinations. Probably the higher temperature during the dry season affected the effectiveness of the rhizobium strain (Piha and Munns, 1987). Unfortunately no soil temperature data were available for the experimental plots during the growing periods for evidence that higher soil

temperature reduced rhizobia efficacy. Nodules were completely absent on roots of snap bean in most of the plots at Ziway and in some plots at Hawassa. The report by Otieno et al. (2009) showed rhizobium inoculation did not increase grain yield of common bean.

The performance of snap bean cultivars for marketable yield was higher at Hawassa under irrigated conditions in general terms. This may be due to the suitability of agro-ecology prevailing at Hawassa for utilizing soil resources for maximum productivity. As indicated in Table 5.2, Contender Blue, Paulista and Volta were among the best performing cultivars for marketable yield at Hawassa. The high productivity of these commercial cultivars under irrigated conditions may be due to availability and frequency of water that allowed in for expression of full genetic yield potential.

The texture and appearance of snap bean pods were improved by application of N fertilizer particularly in some cultivars. This may be due to the fact that good nutrition made green pods well-formed and straight, bright in color and of acceptable quality. The result also showed that applied N improved the texture and appearance of snap bean pods at Debre Zeit and Ziway. Rhizobium inoculation resulted in improved texture and appearance significantly at Debre Zeit. This may indicate that rhizobium inoculation had an effective contribution at Debre Zeit for improving pod texture and appearance.

Commercial cultivars produced high quality pods owing to a fine texture, with well-rounded and straight pods. In contrast, Melkassa cultivars lacked some quality characteristics including smoothness and uniformity of pods. All commercial cultivars were consistently fine in texture across all locations under irrigation. However, the appearance of pods was better when snap bean cultivars were grown at Debre Zeit and Hawassa in general. All cultivars produced pods in the acceptable quality range indicates that snap bean production is possible without N application at all three sites. However, the volume of production was low without N fertilizer.

Applied N reduced TSS but increased TA. The reduction of TSS in response to applied N was also reported by Hozhabryan and Kazemi (2014) in tomato fruit. The TSS of snap bean cultivars was affected by the interaction of cultivar and location but not cultivar alone. The result also indicates that the impact of location highly pronounced the cultivar differences. This result is in agreement with Snodgrass et al. (2011) who reported a no significant differences among

cultivars for TSS. However, certain cultivars produced low TSS at some specific location but this was not consistent from location to location. This may be due environmental variables specific to each location that can determine the performance of a particular cultivar (Hoogenboom, 2000). Commercial cultivars were higher in TA and in general, snap bean cultivars produced at Ziway were lower in TA.

The cultivar by location interaction showed that cultivars produced higher protein and P at Hawassa, particularly Melkassa 3. Pod protein and P concentrations followed no similar trend at Debre Zeit and Ziway. Protein was higher at Ziway but P was higher at Debre Zeit when the two locations were compared numerically.

Andante was numerically the top cultivar in producing total Ca in its pods when grown at Ziway. The result is in agreement with reports of cultivars with smaller pod diameters having higher Ca concentrations (Grusak and Pomper, 1999). The higher soil pH value at Ziway may have also contributed to higher Ca concentrations in pods. The availability of Ca in the soil to plants is affected by H^+ concentration. At higher pH (pH > 7.2), Ca will be more available as the H^+ is lower (Alem and Naqvi, 1999).

Higher K concentration was observed when snap bean was grown at Hawassa but snap bean at Debre Zeit produced lower K than Hawassa and Ziway. This may be due to the fact that exchangeable K concentration in the soil was lower at Debre Zeit but higher at Hawassa and Ziway. This result was supported by Ibrahim et al. (2010) and Mona et al. (2011) who reported that increasing K in the soil increased K concentration in snap and faba bean plants, respectively.

For relative copmarison, the marketable yield obtained under irrigation was higher compared to the yield under rain fed conditions. The marketable yields from Debre Zeit and Ziway under irrigation were comparable with the snap bean yield reported by Elhag and Hussein (2014) from Sudan (24 t ha^{-1}), and Feleafel and Mirdad (2014) from Egypt (21.7 t ha^{-1}). The very high marketable yield obtained from Hawassa under irrigation was resulted from extended harvest time up to five heavy pickings as compared to three pickings under rain fed conditions. The TSS, protein concentration (at Hawassa and Ziway), Zn and K concentrations were almost always greater under irrigation compared to rain fed conditions in general terms.

5.6 Conclusion

Nitrogen fertilizer application at a rate of 100 kg N ha^{-1} increased marketable yield by 33% as compared to the unfertilized treatment. Applied N also improved other quality characteristics including texture, appearance and TA of snap bean pods. Biological N_2 fixation was not effective in increasing marketable yield and most other physical qualities of snap bean pods under irrigation. However, the texture of both Melkassa cultivars and the appearance of Melkassa 1 were improved by N_2 fixation. Melkassa 3 was a better cultivar for producing higher protein, P and K concentrations, particularly at Hawassa. Nitrogen treatment and cultivars interacted with location to affect texture and appearance of snap bean pods. Generally, Hawassa was the site with the greatest marketable yield, protein, K and Zn concentrations in snap bean pods.

5.7 Prologue to chapter 6

In chapters 3 and 4, it is confirmed that snap bean can be produced for export and local markets under rain fed conditions provided that adequate water is available to produce quality pods. The yield and quality obtained under rain fed conditions were also compared with the results in chapter 5 which was investigated under irrigated conditions. Because bean growing regions in eastern Africa are drought prone, drought may occur at any time during plant growth that may affect pod yield and quality. A study in chapter 6 was conducted to determine the effect of drought stress during different developmental stages on pod yield, quality and nutrient concentrations of snap bean.

CHAPTER 6

6. Effect of Temporary Drought Stress at Different Developmental Stages on Pod Yield, Quality and Nutrient Concentrations of Snap Bean Cultivars under Greenhouse Conditions

6.1 Abstract

Snap bean (*Phaseolus vulgaris* L.) for export and local markets can be produced under rain fed conditions provided that adequate moisture is available to produce quality pods. However, drought may occur at any stage during the lifecycle (or growing season) of snap bean. There is a lack of information on the physical quality and nutrient concentrations of snap bean in response to drought stress at different developmental stages. The objectives of this study were: 1) to evaluate the impact of drought stress at different growth stages on physical pod quality; 2) to evaluate the influence of drought stress on zinc (Zn), iron (Fe), protein, Calcium (Ca), phosphorus (P) and potassium (K) concentrations of snap bean pods; and 3) to evaluate the relative tolerance of snap bean cultivars to drought stress at different growth stages in order to identify the most sensitive stage. The experiment was conducted from September to November in 2012 and from October to December in 2013, at the Horticulture greenhouse, Hawassa University, Ethiopia. Drought stress (50% of field capacity) was applied at three different developmental stages: the fourth trifoliate leaf has unfolded (V4.4), flowering (R6) and pod formation (R7) and a control treatment with no stress. Eight cultivars were used and factorially arranged as a completely randomized design with three replications. Our result showed that drought stress during reproductive stages (R6 and R7) caused quality deterioration. Drought stress increased protein, P and Zn concentration but it reduced Fe concentrations in snap bean pods. Ultimately drought stress adversely decreased nutrient content of snap bean pods. All cultivars had a similar response to drought stress.

Key words: Snap bean; Drought; quality; Nutrient

6.2 Introduction

Inadequate and variable water supply has a negative impact on crop production across different climatic regions. The problem is more pronounced in tropical and subtropical semiarid and arid climates in which water losses through evaporation and evapotranspiration are very high throughout the year (Perry and Perry, 1989). Management of water resources is a much greater and more universal problem than any other environmental factors (Mavi and Tupper, 2004).

Water is the most important factor in determining the growth and development of snap bean (*Phaseolus vulgaris* L.). Drought may occur at any stage during growth (Acosta-Gallegos and Shibata, 1989). Drought contributes to reduced yield and poor quality of snap bean pods. A previous report indicated that drought reduced the number of flowers, pod setting and leaf area in bean (Barrios et al., 2005). Drought stress at flowering (Calvache et al., 1997; Manjeru et al., 2007) and after flowering (Manjeru et al., 2007) demonstrated these were the most sensitive stages with the lowest water use efficiency (Calvache et al., 1997), resulting in low yield (Manjeru et al., 2007) in common bean. Another study in bean showed that drought stress during preflowering and flowering stages reduced yield and quality in both these stages (Gunton and Evenson, 1980). According to Cakir (2004) drought stress at all growth stages reduced yield and yield components of maize (*Zea mays*) but the effect was worst when drought occurs during reproductive stage. Ghassemi-Golezani et al. (2009) reported that drought stress at all stages of growth reduced grain yield of faba bean (*Vicia faba*) cultivars.

In Sub Saharan African countries, including Ethiopia, moisture is scarce for dryland agriculture due to more frequent droughts. Snap bean can be produced for export and local markets under rain fed conditions provided that adequate water is available to produce quality pods. This fact is confirmed by the results in the previous chapters (Chapter 3 and 4). However, drought may occur at any time during plant growth that may affect pod yield and quality. The extent of damage from drought among different cultivars of snap bean is not known. Similarly, information is not available on the physical quality and nutrient concentrations of snap bean in response to drought stress at different developmental stages. Information generated from this study will be useful to develop appropriate irrigation schedules for snap bean either for irrigated or supplemental irrigation to rain fed production. Further, this research is very important in

determining planting time of snap bean cultivars under natural rainfall conditions in specific regions in order to avoid stress during sensitive growth stages.

The objectives of this study were: 1) to evaluate the impact of drought stress at different growth stages on physical pod quality; 2) to evaluate the influence drought stress on zinc (Zn), iron (Fe), protein, calcium (Ca), phosphorus (P) and potassium (K) concentrations of snap bean pods; and 3) to evaluate the relative tolerance of snap bean cultivars to drought stress at different growth stages and to identify the most sensitive stage of development to drought stress.

6.3 Materials and Methods

6.3.1 Experimental design and application of treatments

The current experiment was conducted from September to November in 2012 and from October to December in 2013 at the Horticulture greenhouse, Hawassa University, Ethiopia. Seeding was done on 20th September and 30th October in 2012 and 2013, respectively. Field soil from the Research and Farm Center at Hawassa University was used for the experiment. Temperature and relative humidity in the greenhouse are presented on Appendix 13. The soil was characterized for major physicochemical characteristics (Appendix 2).

The percentage of moisture content at field capacity (%FC) of the soil was determined gravimetrically (Reynolds, 1970). Drought stress was set as 50% of the FC because this was found to be sufficient to stress snap bean in preliminary research. Drought stress was applied at three different developmental stages: 1) the fourth trifoliate leaf (the stage begins when the fourth trifoliate leaf on the stem of 50% of the plants in a bean crop is unfolded [V4.4]); 2) flowering (this stage begins when the first open flower appears on the plant or when 50% of the plants in a bean crop have an open flower [R6]); and 3) pod formation (this begins when a plant shows the first pod with the flower's corolla hanging or detached and in a crop when 50% of the bean plants show this characteristic [R7]) as defined by CIAT (1986). A control treatment (continuous watering throughout the growth period maintaining moisture level above 90% FC) was included as a fourth treatment. The four treatments were combined with eight snap bean cultivars (Andante, Boston, Conteder Blue, Lomami, Melkassa 1, Melkassa 3, Paulista and Volta). The drought stress treatments and the cultivars were factorially applied as a completely randomized design (CRD) with three replications. Each treatment consisted of four pots sown

with four seeds in each pot and thinned to two plants per pot at the primary leaf stage (V2). Pot size was 7 l in volume and each pot was filled with three kg of dry soil. At each specified growth stage, the moisture level was maintained at 50% FC for 5 days by weighing and adding the deficit water when the wet weight of the pot (pot plus soil plus plant) decreased below 50% of FC each day. Because cultivars had different days to maturity, drought treatments were applied at equivalent development stages.

6.3.2 Measurements

6.3.2.1 Pod yield and maturity

Days to maturity was calculated as the number of days from planting to 50% harvest maturity. Optimum maturity was considered when pods were firm with green young seeds, before seeds pushed out the pulp visibly. Generally, snap bean reached at optimum maturity between 17 - 20 days after flowering under greenhouse conditions. The fresh weight of the pods from all the four pots at optimum maturity was recorded with a sensitive balance and the average yield weight was calculated per plant. Pod number per pot and average pod number per plant were measured. The dry weight of the pods was determined by drying the pods in an oven for 48 h at 70°C. After cooling to room temperature, the samples were weighed.

6.3.2.2 Pod quality data

Pods from all the four pots were harvested and the marketable pods (defectless quality pods) were sorted at optimum maturity. The fresh weights of marketable pods were taken and calculated as marketable pod per plant. Pod quality parameters of length, diameter, total soluble solids (TSS), titratable acidity (TA), curvature, and nutrient concentrations (protein, P, Zn, Fe, Ca and K) were determined using the same method reported in Chapter 4 (4.3.3).

6.3.3 Statistical analysis

For data analysis, the PROC MIXED procedure of 9.3 SAS software (SAS Institute Inc. 2012) was used. The two years of data from this greenhouse experiment were combined. Means were separated according to Fisher's protected LSD at $P \leq 0.05$. The *P*-values from mixed model ANOVA F-test Tables for the response variables are presented on Appendices 14, 15 and 16.

6.4 Results

6.4.1 Pod yield and maturity

Total yield, number of pods and days to maturity were affected by drought stress at different developmental stages and cultivars. Pod dry weight per plant was only affected by drought stress.

Drought stress during all developmental stages reduced total yield per plant of snap bean (Table 6.1). The drought treatment during R6 and R7 reduced total yield by 36% (Table 6.1). All cultivars had the same total yield per plant except Andante which was significantly lower (Table 6.1).

Even though drought stress during all developmental stages reduced number of pods per plant, pod reduction was most severe at R6 (Table 6.1). The impact of drought stress during all developmental stages was similar for pod dry weight per plant (Table 6.1). Andante produced a greater number of pods per plant comapred to other cultivars except Boston and Lomami (Table 6.1). Melkassa cultivars produced number of pods per palnt compared to commercial cultivars (Table 6.1).

Drought stress at all developmental stages delayed maturity of snap bean pods and the delay was most pronounced when drought stress occurred at V4.4 and R7 (Table 6.1). Melkassa 1 was the latest cultivar to mature and Andante was earlier maturing than all other cultivars except Contender Blue (Table 6.1).

Table 6.1. Effect of temporary drought stress applied at different developmental stages on means of total yield per plant, number of pods per plant, pod dry weight per plant and days to maturity for eight snap bean cultivars

	Total yield plant^{-1}	Number of pods plant^{-1}	Pod dry weight plant^{-1}	Days to maturity
	g		g	days
Drought stress				
Control	35.1a	13.9a	2.96a	50.7c
V4.4	26.7b	11.4b	2.09b	53.1a
R6	22.4c	9.65c	1.89b	51.5b
R7	22.3c	10.9b	2.04b	53.1a
Cultivar				
Andante	21.9b	13.6a	2.09	51.0e
Boston	28.9a	13.1ab	2.3	52.8b
Contender Blue	25.6a	11.4d	2.19	51.5de
Lomami	28.0a	12.8abc	2.32	52.4bc
Melkassa 1	29.1a	9.56e	2.24	53.8a
Melkassa 3	26.0a	7.99f	2.06	51.8cd
Paulista	26.5a	11.6cd	2.20	51.9cd
Volta	27.1a	11.9bcd	2.55	51.6d

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P \leq 0.05$ Absence of a letter in a grouping column denotes non significance.

6.4.2 Pod marketable yield other physical qualities

Drought stress during different developmental stages for the eight cultivars significantly affected marketable yield, length, diameter, texture and appearance of snap bean pods. But cultivar and drought stress had no effect on TSS and TA of snap bean pods.

Drought stress during V4.4, R6 and R7 stages reduced marketable yield by 25%, 42% and 48%, respectively, (Table 6.2). Lomami produced a higher marketable pod yield than Andante and Contender Blue (Table 6.2). Drought stress at all developmental stages reduced pod length but pod diameter was reduced only when drought stress occurred during R6 (Table 6.2). The longest pods were produced by Melkassa 3 and the largest pod diameter was produced by Melkassa 1 (Table 6.2). Andante produced the shortest pods and smallest pod diameter (Table 6.2).

The most curved pods were produced under drought stress during R6 followed by R7 (Table 6.2). Drought stress during V4.4 had no impact on curvature of pods (Table 6.2). Cultivar differences were not evident for pod curvature.

A coarser texture and poorer appearance were recorded under drought stress during R6 and R7 stages (Table 6.2). Drought stress during V4.4 had no effect on the texture of snap bean but resulted in a poorer appearance of pods compared to the control (Table 6.2). The texture and appearance of Melkassa cultivars were coarser and poorer than other commercial cultivars (Table 6.2).

Table 6.2. Mean marketable pod yield per plant, pod length, pod diameter , pod curvature, texture and appearance of eight cultivars of snap bean as affected by temporary drought stress at different developmental stages

	Marketable yield plant^{-1}	Pod length	Pod diameter	Pod curvature	Texture	Appearance
	g	mm	mm		(1-5)†	(1-5)‡
Drought stress						
Control	32.8a	112a	6.48a	0.980a	1.54b	1.65d
V4.4	24.5b	104b	6.45a	0.980a	1.56b	1.87c
R6	19.0c	105b	6.25b	0.860c	3.15a	3.71b
R7	17.2c	102b	6.44a	0.900b	3.29a	3.94a
Cultivar						
Andante	19.8c	95e	5.12e	0.940	2.17c	2.46cd
Boston	26.2a	106bc	6.19cd	0.936	2.21c	2.42d
Contender Blue	22.4bc	99cd	6.36c	0.932	2.13c	2.63cd
Lomami	25.1ab	105c	6.00d	0.933	2.21c	2.50cd
Melkassa 1	24.7ab	109b	7.87a	0.935	3.17a	3.75a
Melkassa 3	22.8abc	118a	7.13b	0.928	2.70b	3.33b
Paulista	23.2abc	105c	6.38c	0.915	2.33c	2.75c
Volta	23.1abc	109b	6.21cd	0.930	2.08c	2.50cd

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P \leq 0.05$. Absence of letter in a grouping column denotes non significance.

†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)
‡Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)

6.4.3 Pod nutrient concentrations

Drought stress significantly affected all nutrient concentrations considered and cultivar differences significantly affected protein, Zn, Fe and Ca concentrations in snap bean pods.

Drought stress during all developmental stages significantly increased pod protein concentration (Table 6.3). Drought stress during reproductive stages (R6 and R7) increased protein concentration more than drought stress during vegetative stage (V4.4) (Table 6.3). The results showed that protein concentration had highly and significant negative correlations with total yield (r = -0.99**), marketable yield (r = -0.99**) and Fe (r = -0.98*). In contrast, protein had highly and significant positive correlations with Zn (r = 0.98*) concentration. With regard to P, drought stress during flowering stage (R6) significantly increased P concentration compared to the unstressed treatment (Table 6.3). Amongst cultivars, Lomami produced a greater protein concentration than Andante, Contender Blue and Melkassa cultivars (Table 6.3).

Drought stress during all developmental stages increased Zn concentration in snap bean pods (Table 6.3). Drought stress during reproductive stages (R6 and R7) significantly increased Zn concentration compard to drought stress at V4.4 (Table 6.3). Zinc concentration had highly and significant negative correlations with total yield (r = -0.96*), marketable yield (r = -0.98*) and Fe (r = -0.99**). Amongst cultivars, Melkassa 1 had a greater Zn concentration compared to Contender Blue, Melkassa 3, Paulista and Volta under greenhouse conditions (Table 6.3).

Drought stress during all developmental stages reduced Fe concentration of snap bean pods (Table 6.3). Severe reduction of Fe was observed when drought stress occurred during reproductive stages (R6 and R7) (Table 6.3). Iron concentration had a highly and significant positive correlation with total yield (r = 0.97*) and marketable yield (r = 0.99**). Contender Blue had a greater pod Fe concentration than Andante, Lomami, Paulista and Volta (Table 6.3).

Drought stress during V4.4 and R6 tended to increase Ca concentration (Table 6.3). Andante, Boston, Paulista and Volta had a greater Ca concentration in their green pods than the other cultivars (Table 6.3). Drought stress during R6 stage increased K concentration of pods (Table 6.3), but there was no significant difference among cultivars (Appendix 16).

Table 6.3. Protein, phosphorus (P), zinc (Zn), iron (Fe), calcium (Ca) and potassium (K) concentrations of snap bean pods as affected by temporary drought stress at different developmental stages and cultivar

	Protein	P	Zn	Fe	Ca	K
	%	%	ppm	ppm	%	%
Drought Stress						
Control	19.13c	0.479b	33.4c	130a	0.60ab	3.57b
V4.4	20.19b	0.493ab	35.3b	122b	0.63a	3.67b
R6	21.05a	0.518a	37.9a	114c	0.62a	3.90a
R7	20.99a	0.508ab	38.4a	111c	0.57b	3.57b
Cultivar						
Andante	19.68c	0.509	37.9ab	104c	0.68a	3.67
Boston	21.08ab	0.492	36.4abc	128a	0.68a	3.69
Contender Blue	19.80c	0.489	35.5bc	133a	0.56b	3.69
Lomami	21.20a	0.510	36.7abc	105bc	0.56b	3.77
Melkassa 1	20.05bc	0.486	38.5a	130a	0.55b	3.50
Melkassa 3	20.02bc	0.498	35.4bc	129a	0.57b	3.63
Paulista	20.09abc	0.505	35.3c	115b	0.63a	3.70
Volta	20.78abc	0.507	34.2c	111bc	0.64a	3.76

Means followed by the different letters in a treatment grouping column differ significantly based on LSD, $P \leq 0.05$. Absence of letter in a grouping column denotes non significance

% Determined on the basis of g 100 g^{-1} of pod dry weight.
ppm=µg g^{-1} of pod dry weight

No drought stress | Drought stressed during V4.4

Drought stress during R6 | Drought stress during R7

Fig. 6.1. Pods of snap bean cultivar (Paulista) under drought stress during different developmental stages

6.5 Discussion

Drought stress at all developmental stages reduced yield and yield components of snap bean pods under greenhouse conditions. The yield reduction was severe when drought occurred during the reproductive phases. Previous reports on common bean confirmed this result (Nielsen and Nelson, 1998; Boutraa and Sanders, 2001; Molina et al, 2001). Pod number per plant was reduced when drought stress occurred during flowering (R6). This reduced pod number may be due to abscission of reproductive organs (flowers and pods) caused by drought (Boutraa and Sanders, 2001).

Drought stress during vegetative (V4.4) and reproductive stages (R6 and R7) delayed maturity of snap bean. Delayed maturity due to drought during vegetative stage may be due to low assimilation by stressed plants that resulted in a slow growth rate (Boonjung and Fukai, 1996). Drought stress during V4.4 stage would reduce photo-assimilation capacity by reducing leaf area for photosynthesis. A slight but non-significant delay in maturity of common bean in drought stress occurring in flowering and pod development was reported by Boutraa and Sanders (2001). Contrast to the current result, Rosales-Serna et al. (2004) reported that terminal drought accelerated maturity of common bean cultivars under field conditions.

Yield and yield component differences among cultivars were mainly associated with pod size and plant size. Small cultivars with small pod diameters and length such as Andante had lower yield. Both pod size (length and diameter) and number determined the yield of a cultivar. A higher number of pods may not necessarily lead to higher yield because a smaller number of pods of larger size may result in greater yield than a large number of pods of smaller size. This is reflected in Andante which had lower yield due to small pod size and in Melkassa 1 which had larger size pods but they were few in numbers. These two cultivars also contrasted in plant size and days to maturity, which Andante was smaller and earlier to mature compared to Melkassa 1.

Drought stress during reproductive stages (R6 and R7) markedly reduced marketable yield by 42 and 48%, respectively. In addition to the direct impact on yield, drought also affected marketable yield indirectly by reducing the appearance of pods with curved, malformed and undeveloped pods which were not marketable (Fig. 6.1). Drought stress during V4.4 also reduced marketable yield but the relative difference was small. This indicated that drought at

V4.4 stage affected the marketable yield by reducing the amount of yield and only slightly affected pod appearance (Fig. 6.1).

Drought stress at all three developmental stages reduced pod length. The most curved pods resulted from drought stress during flowering (R6). Drought stress during reproductive stage (R6 and R7) resulted in curved pods with rough texture and poor appearance. The yield reduction of common bean due to drought stress is well documented. However, there is no report on the extent of curving of pods due to drought stress.

Drought stress during all developmental stages increased protein and Zn concentrations but decreased Fe concentration. In this experiment Fe concentration was negatively correlated with protein and Zn in response to a short drought stress. Increased or decreased levels of protein in response to drought stress depend on plant species and organ (Sharma and Dubey, 2011). Drought stress increased protein concentrations in chickpea shoot (Rai et al., 1983), the whole barley plant (Bole and Pittman, 1978), in tomato leaves (Chao et al., 1999), and the alfalfa shoot (Aranjuelo et al., 2011). However, levels of protein were reduced in immature and mature pods of broad bean (Ouzounidou et al., 2014), leaves of common bean (Lazcano-Ferrat and Lovatt, 1999), the wheat plant (Kulshrestha et al., 1987) and in shoots and roots of rice (Sharma and Dubey, 2005). Reports are contradictory for common bean seed protein concentration in response to drought stress. According to De Mejia et al. (2003) common bean seed protein increased in response to drought. In contrast, Ghanbari et al. (2013) reported decreased seed protein under drought stress. Ghanbari et al. (2014) reported an increased trend of protein accumulation in developing seeds of common bean but a significantly reduced protein concentration at final seed development. Previous report indicated that the nitrogenous solutes and free amino acids are accumulated in plant tissue in response to moisture deficit, but soluble proteins decreased (Handa et al., 1983). The increase in protein concentration in pods in the current experiment may be due to higher accumulation of N solutes and free amino acids under drought stress. Ghanbari et al. (2014) also reported significant reductions in Fe and Zn due to drought stress in seeds. Another report indicated increased Zn concentration in response to increased drought stress in alfalfa shoot (Kidambi et al., 1990). Our results were in agreement with respect to reduced Fe concentration but contrasted with reduced Zn. Our results showed an increased Zn concentration in snap bean pods due to drought stress. Reports suggest that plant

mineral (iron and Zn) concentrations vary with plant tissue (Nchimbi-Msolla and Tryphone, 2010). Zn concentration in the pods showed similar pattern with protein concentration under drought stress. This result is not unexpected because Zn plays a significant role in the activation of enzymes and protein synthesis (Cakmak et al., 1989). Iron availability in plant tissue depends upon its availability in soil, rate of absorption by the plant root, reduction of Fe^{3+} to Fe^{2+} in the apoplast of the root and Fe^{2+} uptake from the apoplast into the cytosol (Mengel, 1994). Drought stress may negatively affect these processes that lead to lower Fe accumulation in the pods of snap bean. Iron absorption is affected by rate of absorption and translocation into the plant system. A lot of Fe absorption and translocation also takes place late in the season, so late drought resulted in dramatic reduction of Fe concentration in the plant tissue. However, the specific mechanism how drought stress reduced Fe concentration in pods needs further investigation.

Our patterns of P and K concentrations showed a similar response to drought. Both nutrients concentrations were greater due to drought at flowering (R6). Report indicated that drought stress increased the concentration of K but decreased P (Kidambi et al., 1990). However, to the best of our knowledge, there is no report on P and K concentrations in pods as a result of drought stress, particularly during flowering (R6). However, the increase in nutrient concentration is at the expense of yield. Protein content per plant was decreased by 25, 30 and 24% due to drought stress at V4.4, R6 and R7 developmental stages, respectively. Zinc content also decreased by 25, 27 and 21% due to drought stress at V4.4, R6 and R7 developmental stages, respectively. Overall, this showed that drought stress had a negative impact on nutrient content of snap bean pods because drought reduced number and size of pods (yield).

6.6 Conclusions

All cultivars considered in this experiment had a similar response to drought stress during the three developmental stages. Drought stress during reproductive stages (R6 and R7) had the greatest effect in deteriorating the quality of snap bean pods. Drought stress during R6 resulted in the most curved pods. Drought stress during V4.4 stage reduced yield but had little impact on pod quality parameters such as curvature and texture of pods. Generally, a short drought stress under greenhouse conditions increased protein, P and Zn concentrations. However, it reduced Fe concentration in snap bean pods. Additionally, drought stress during flowering increased K

concentration. The increase in nutrient concentration was at the expense of yield. Therefore, drought stress adversely decreased nutrient content of snap bean pods.

6.7 Prologue to chapter 7

In chapter 6 greenhouse experiment, drought stress at various developmental stages reduced yield, physical quality and nutrient content of snap bean pods. However, drought increased protein and Zn concentrations. Drought stress during reproductive stage (R6 and R7) severely deteriorated the pod quality of snap bean. The most curved pods were produced when droght stress occurred during R6. Better quality snap bean pods can be produced if the impact of drought stress is reduced. A study in chapter 7 was conducted to test whether foliar applications of growth regulators; abscisic acid, kinetin, salicylic acid and yeast extract can reduce the impact of drought stress on snap bean yield and pod quality.

CHAPTER 7

7. Reducing the Impact of Drought Stress on Snap Bean Yield and Quality through Application of Plant Growth Regulators

7.1 Abstract

Pod quality of snap bean (*Phaseolus vulgaris* L.) can be severely deteriorated due to drought stress, particularly when stress occurs during reproductive growth. Growth regulators can be used in crop management to improve water use efficiency of crops. Therefore, our study aimed at testing whether foliar application of growth regulators (abscisic acid [ABA], kinetin, salicylic acid [SA] and yeast extract) can reduce the impact of drought stress on snap bean yield and pod quality. An experiment was conducted twice from February to June 2014 in the greenhouse, Hawassa University, Ethiopia. The experiment combined two levels of drought treatments (unstressed and drought stress during flowering) and nine levels of growth regulator treatments as foliar applications. The drought stress was 50% of field capacity during flowering for 5 days. The growth regulator treatments were control, 10^{-5} and 10^{-4} M concentrations of each of ABA, kinetin and SA; and two concentrations of yeast extract (4 and 8 gram per liter).The two factors were factorially applied as a Completely Randomized Design (CRD) with four replications. Foliar application of SA on snap bean reduced the impact of drought stress, particularly in pod marketable yield, curving, texture and appearance of the pods. However, application of ABA, kinetin and SA deteriorated pod quality of snap bean under unstressed conditions. Our results showed that judicious application of SA could reverse the negative impact of drought stress on quality of snap bean pods.

Key words: snap bean; quality; growth regulator; drought

7.2 Introduction

Drought is a highly complex phenomenon that exerts a deleterious impact on crop survival, productivity and quality of yield. The issue of drought needs research to tackle problems associated with yield reduction and quality deterioration. Earlier studies on drought in crops were targeted to water saving mechanisms by plants as an objective for breeding and management practices to solve drought related problems (Acquaah, 2012). However, this

concept has proven to be inadequate in addressing moisture deficit problems in modern agriculture (Acquaah, 2012). Rather, efficient water use is more important than water saving mechanisms in most crops because water is critical to sustain yield (Acquaah, 2012).

Several studies have showed that there is a decrease in concentrations of auxin, cytokinin (CK) and gibberellic acid (GA_3) and an increase the level of abscisic acid (ABA) during drought stress in many plants (Figueiredo et al., 2008; Sadeghipour and Aghaei, 2012; Yurekli et al., 2004; Farooq et al., 2009a). Plant hormones can be managed through their chemical analogys or unrelated chemicals that control growth and development at lower concentration. Some plant growth regulators can improve water use efficiency in plants. There is sufficient evidence for the involvement of ABA in mediating drought stress tolerance of crops (Bray, 1993; Yin et al., 2004). However, an increase in ABA during drought stress frequently results in a negative impact on plants such as flower and fruit abscission, and finally reduced yield and quality (Artlip et al., 1995; Yin et al., 2004).

Studies indicated that CK is involved in drought tolerance of many plants (Metwally et al., 1997; Hare et al., 1997; Thomas et al., 1992; Pospisilova et al., 2005; Zhang and Ervin, 2004; Pospisilova et al., 2000). However, there is lack of information on whether exogenous application of CK can positively affect the yield and quality of snap bean under drought stress conditions. Studies have also indicated that yeast extract can reduce flower and fruit abscission, increase yield and tissue nutrients concentrations, and improve the quality of the produce under normal conditions (Shehata et al., 2012; Fawzy et al., 2012; Asmaa et al., 2013; El-Tohamy and El-Greadly, 2007; Mady, 2009). However, its effect under drought is not known. Because yeast extract is a natural and an inexpensive precursor for CK, we expect that yeast extract may reduce the impact of drought.

Many reports indicate that salicylic acid (SA) is also involved in drought tolerance of many plants (Kabiri et al., 2012; Hayat et al., 2012; Habibi, 2012; Sadeghipour and Aghaei, 2012). The impact of exogenous application of SA on quality and nutrient concentrations of snap bean is not well studied and was therefore included in our research.

In our previous results from field experiments, we concluded that snap bean can be produced under low input production systems under dryland agriculture, and can be used by small-scale resource-limited farmers (Chapter 3 and Chapter 4). Additionally, from our

greenhouse experiment (Chapter 6), drought stress at various developmental stages reduced yield, physical quality and nutrient content of snap bean pods. Our experiment further showed that the impact of drought stress was more severe when stress occurred during reproductive growth. Because drought may occur at any time during the growing season, there is a need to develop a strategy that can reduce the impact of stress on yield and quality. Therefore, our study aimed at testing whether foliar applications of growth regulators, ABA, kinetin, SA and yeast extract can reduce the impact of drought stress on snap bean yield and pod quality.

7.3 Materials and methods

7.3.1 Experimental design and application of treatments

An experiment was conducted twice from February to June 2014 in the Horticulture greenhouse, Hawassa University, Ethiopia. Seeding was done on 13th February for the first run, and on 4th April, 2014 for the second run. All crop management activities, determination of soil moisture content, number of pots per treatment, amount of soil per pot and number of plant per pot were similar to the previous greenhouse experiment presented in Chapter 6 (6.2.1). Temperature and relative humidity in the greenhouse and major physicochemical characteristic of the experimental soil are presented on Appendices 17 and 18, respectively.

The experiment combined two levels of drought treatments (unstressed, and drought stress during flowering) and nine levels of growth regulator treatments applied as foliar sprays. The drought stress was 50% of field capacity (FC) during flowering for 5 days. The growth regulator treatments were control, 10^{-5} and 10^{-4} M concentrations of each of ABA, kinetin and SA; and two concentrations of yeast extract, 4 and 8 gram per liter (g l^{-1}). The two factors were factorially applied as a completely randomized design (CRD) with four replications. Detectable hormonal composition of yeast extract is presented in Appendices 22 and 23. The growth regulators were obtained from SIGMA-ALDRICH, Canada. Yeast extract is the water-soluble portion of autolyzed yeast, often used in culture media. Yeast extract contains a mixture of amino acids, peptides, water soluble vitamins and carbohydrates. Detailed composition of yeast extract was determined by Mahmoued (2001) as cited in El-Yazied and Mady (2012). Abscisic acid, kinetin and SA were dissolved in methanol, sodium hydroxide and ethanol, respectively, before making the solution. A stock solution was prepared for each growth regulator. The stock

solution was diluted to 10^{-5} M and 10^{-4} M concentrations and made into 4 l volumes for spraying all four replicates of each treatment. Yeast extract solutions were made by directly solubilizing the yeast in water and made up to 4 l volumes for all four replicates of each treatment. Four l of distilled water was used to spray the replicates of the control treatment. The replicates of a specific treatment were collected together in spray room and sprayed with the solution when drought stress commenced (50% FC). Cultivar paulista was used for conducting this experiment. Paulista is widely planted snap bean cultivar by many commercial snap bean producers in Ethiopia.

7.3.2 Measurements

Data were collected from the same methodology as the previous experimental chapters. Yield parameters were total yield, pod number and pod dry weight (Chapter 6.3.2.1); pod quality parameters were marketable yield (Chapter 6.3.2.2), length, diameter, curvature, texture and appearance of pods, TSS and acidity (Chapter 4.3.3.1); and nutrient concentrations were protein, P, Ca, K, Zn and Fe (Chapter 4.3.3.2).

7.3.3 Statistical analysis

For data analysis, the PROC MIXED procedure of 9.3 SAS software (SAS Institute Inc. 2012) was used for analysis of variance. The two years of data were combined, and means were separated according to Fisher`s protected LSD, at $P \leq 0.05$. The *P*-values from mixed model ANOVA F-test Tables for the response variables are presented on Appendices 19, 20 and 21.

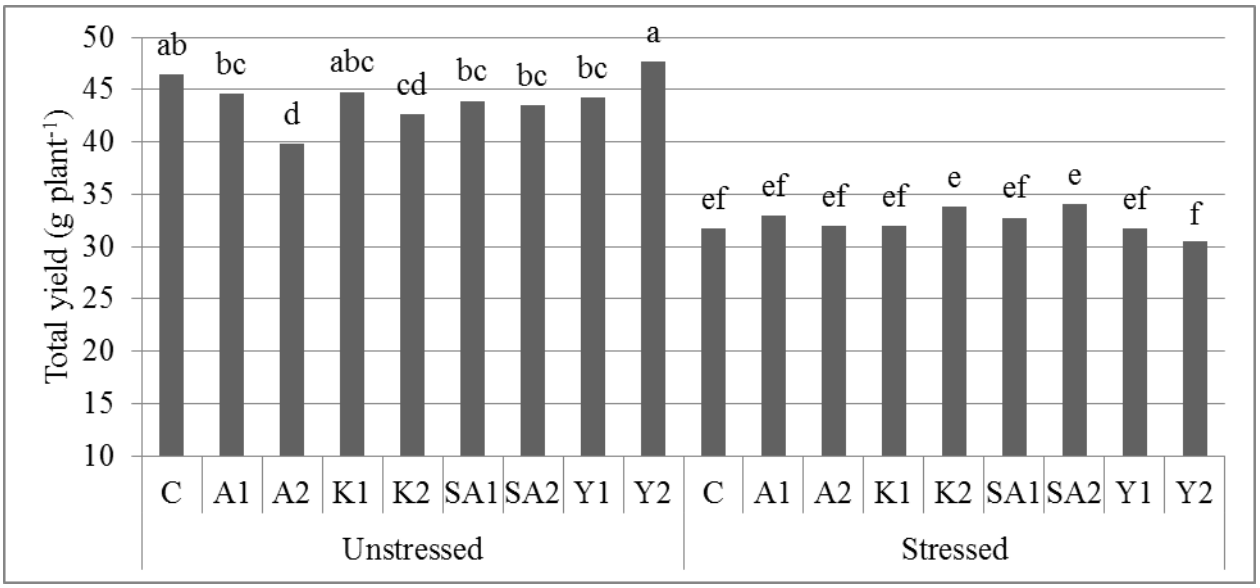
7.4 Results

7.4.1 Yield and yield components

Drought stress and the interaction of drought with growth regulator significantly affected total yield, pod number and pod dry weight per plant. Growth regulators as a main factor had no significant effect on these parameters.

Yeast extract at the higher concentration (8 g l^{-1}) under unstressed conditions resulted in greatest total yield compared to most of other treatment combinations except the control and kinetin at lower (10^{-5} M) concentration (Fig. 7.1). However, it was numerically the least under stressed conditions (Fig. 7.1). Our results indicated that foliar application of growth regulators

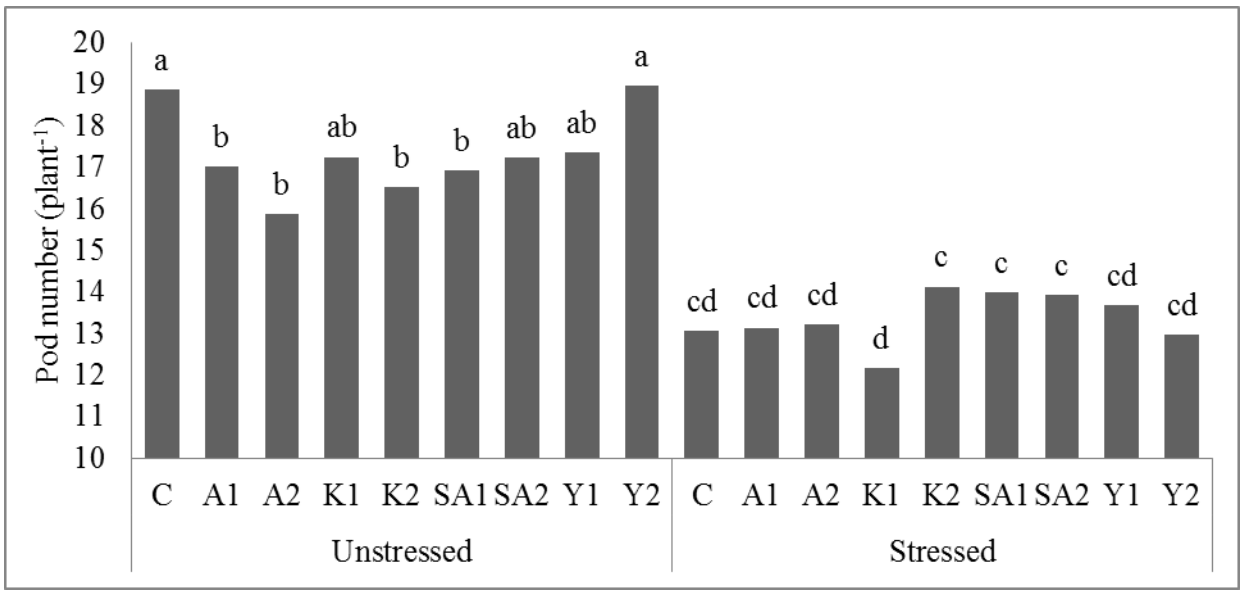
did not reduce the impact of short drought stress on total yield of snap bean (Fig. 7.1). Rather, foliar application of ABA and kinetin at the higher concentration (10^{-4} M) significantly reduced the yield of snap bean pods compared to the control under unstressed conditions (Fig. 7.1).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

Fig. 7.1 Total yield per plant (g) of Paulista snap bean as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

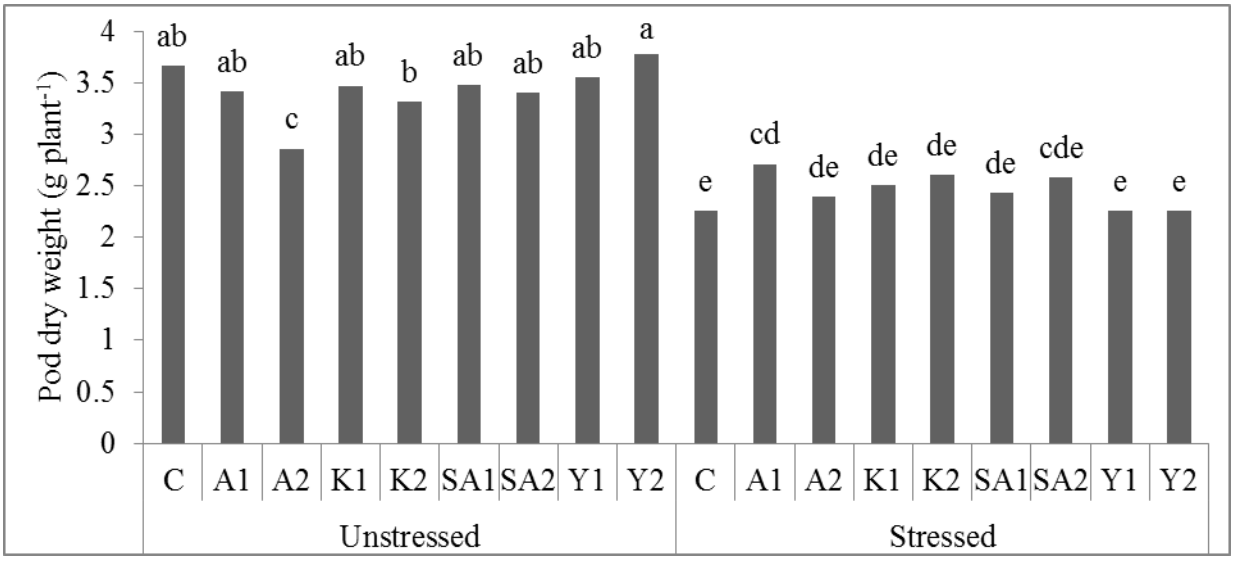
Foliar application of growth regulators did not improve the number of pods per plant under drought conditions (Fig. 7.2). Under unstressed conditions ABA at both lower (10^{-5} M) and higher (10^{-4} M) concentrations, SA at the lower concentration (10^{-5} M) and kinetin at the higher (10^{-4} M) concentration all reduced the number of pods per plant (Fig. 7.2). Yeast extract at the higher concentration and under unstressed conditions resulted in numerically the greatest pod number per plant, but this was not significantly different from the control (Fig. 7.2).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

Fig. 7.2 Pod number per plant as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

Abscisic acid at the lower concentration (10^{-5} M) significantly reduced the impact of the short drought stress during flowering on pod dry weight per plant (Fig. 7.3). Other treatments had no impact in ameliorating drought stress on the dry weight of snap bean pods (Fig. 7.3). Under unstressed conditions, ABA at the higher concentration significantly reduced pod dry weight of snap bean compared to the control (Fig. 7.3).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

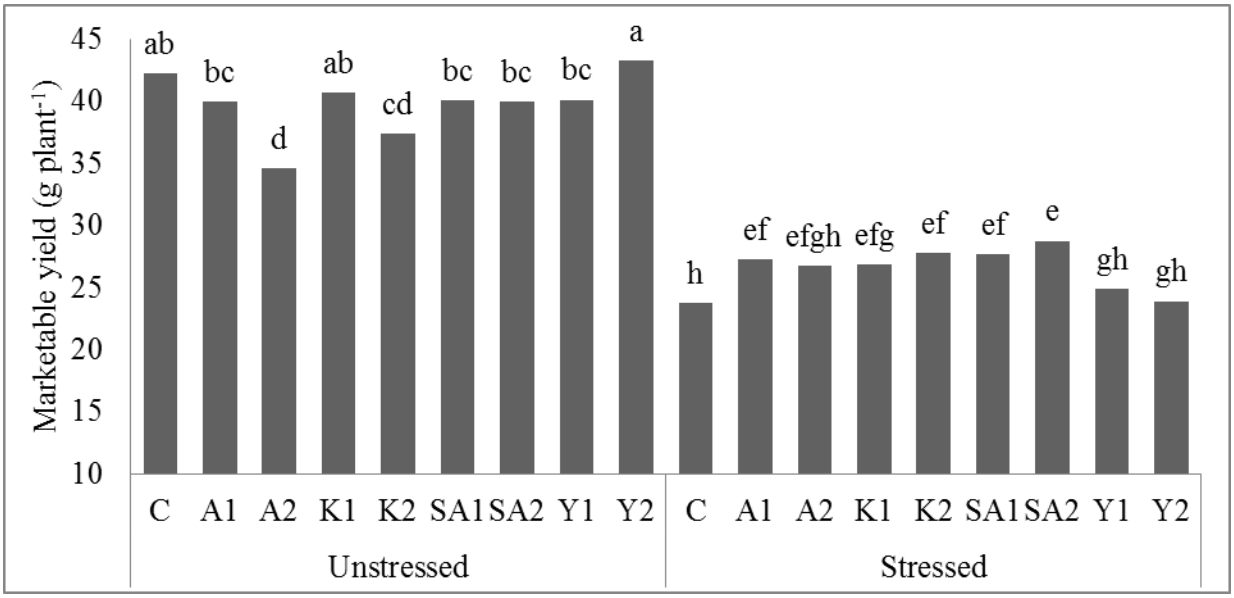
Fig. 7.3 Pod dry weight per plant (g) as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

7.4.2 Pod marketable yield and other physical qualities

The short duration drought stress during flowering significantly affected marketable yield, pod length, curvature, texture appearance and TA of snap bean pods. Growth regulators also significantly affected marketable yield, texture, appearance and acidity of snap bean pods. The interaction of drought stress by growth regulator affected marketable yield, curvature, texture and appearance of snap bean pods.

As a main effect, none of the growth regulators improved the marketable yield of snap bean. Rather, ABA at the higher concentration reduced pod marketable yield. Kinetin and SA at both concentrations and ABA at lower concentration improved marketable yield of snap bean under drought stress conditions only (Fig. 7.4). But under unstressed conditions, ABA and kinetin at the higher concentrations significantly reduced the marketable yield of snap bean

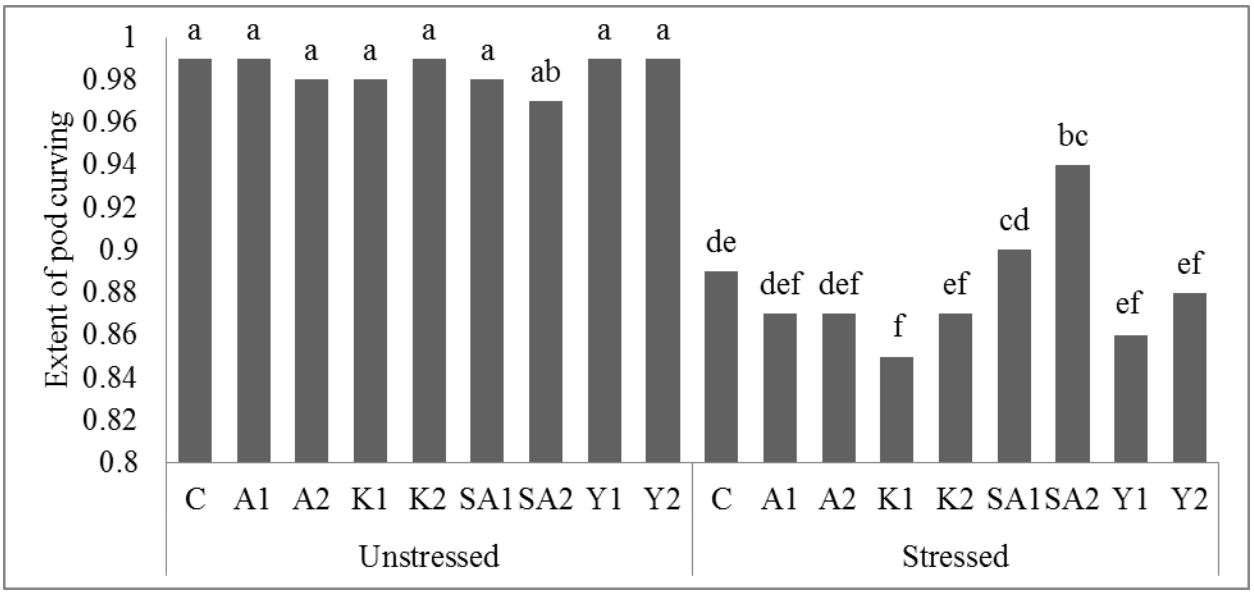
compared to the control (Fig. 7.4). Numerically, maximum marketable yield was obtained when yeast extract was applied at the higher concentration under unstressed conditions (Fig. 7.4).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

Fig. 7.4 Mean marketable yield per plant (g) as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

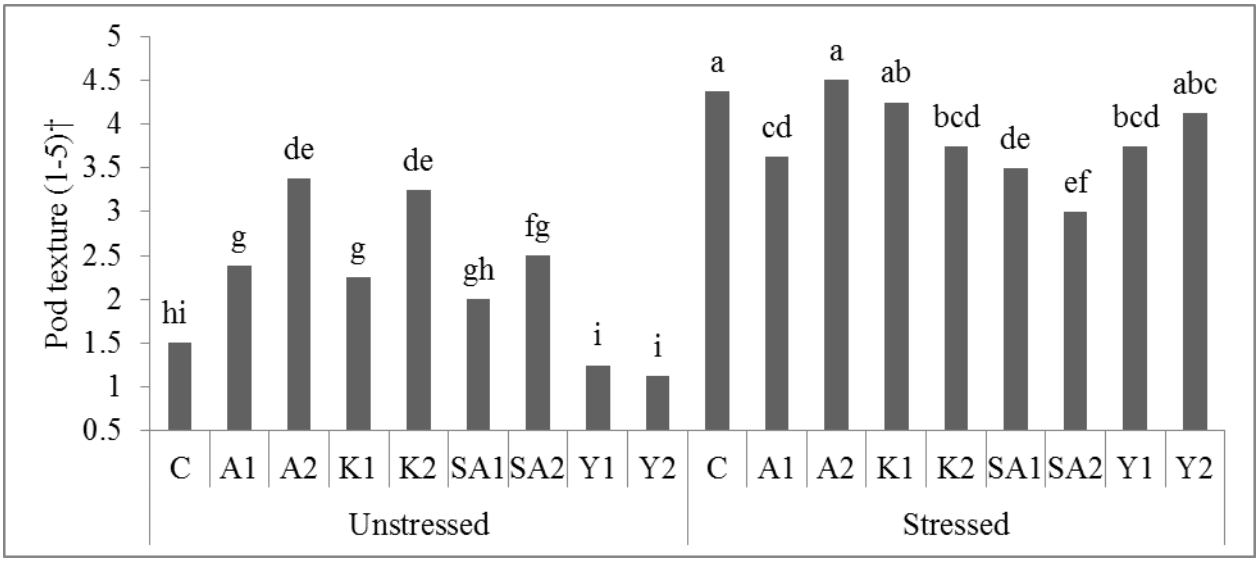
Salicylic acid at the higher concentration (10^{-4} M) reduced the extent of curving of pods under drought stress (Fig. 7.5). Kinetin at lower concentration increased the extent of curving of pods compared to the control under drought (Fig. 7.5), which actually was unexpected. Overall, all the growth regulators had no effect on pod curving under unstressed conditions (Fig. 7.5).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

Fig. 7.5 Pod curvature as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

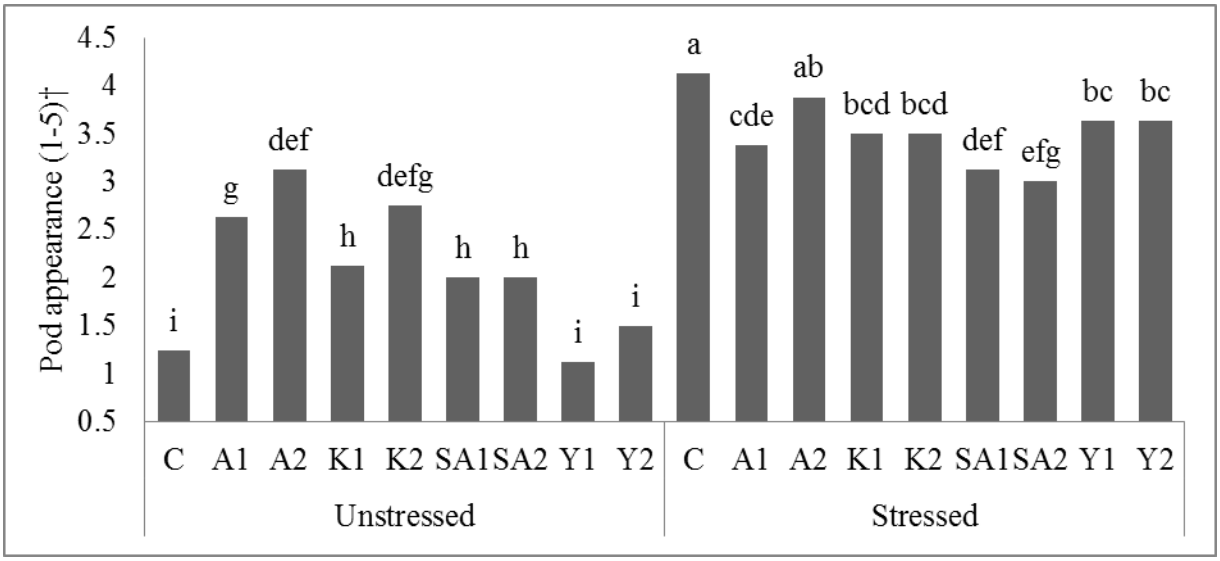
Abscisic acid and yeast extract at lower concentration, kinetin at higher concentration, and SA at both higher and lower concentrations improved the texture of snap bean pods compared to the control under drought (Fig. 7.6). Under unstressed conditions, ABA and kinetin at both higher and lower concentrations and SA at higher concentration worsened the texture of snap bean pods (Fig. 7.6). Numerically, the finest pod texture was obtained from the foliar application of yeast extract at the higher concentration under unstressed conditions. But this was not significantly different from the control (Fig. 7.6).



C=Control K1=10^{-5} M kinetin SA2=10^{-4} M Salicylic acid
A1=10^{-5} M ABA K2=10^{-4} M kinetin Y1=4 g l^{-1} yeast extract
A2=10^{-4} M ABA SA1=10^{-5} M Salicylic acid Y2=8 g l^{-1} yeast extract
†Score (1= very fine, 2 = fine, 3 = reasonably fine, 4 = coarse/ rough, 5 = very coarse/rough)

Fig. 7.6 Pod texture (1-5) as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

Under drought stress conditions all the growth regulators improved the appearance of snap bean pods except ABA at the higher concentration (Fig. 7.7 and 7.8). In contrast, under unstressed conditions, all the growth regulators except yeast extract at both higher and lower concentrations reduced the appearance of snap bean pods (Fig. 7.7). Salicylic acid at both the lower and higher concentrations and ABA at the lower concentration improved the appearance of snap bean pods more than yeast extract, ABA at the higher concentration and the control (Fig. 7.7 and 7.8).



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

†Score (1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = rejected)

Fig. 7.7 Pod appearance (1-5) as a result of drought stress by growth regulator interaction. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$.

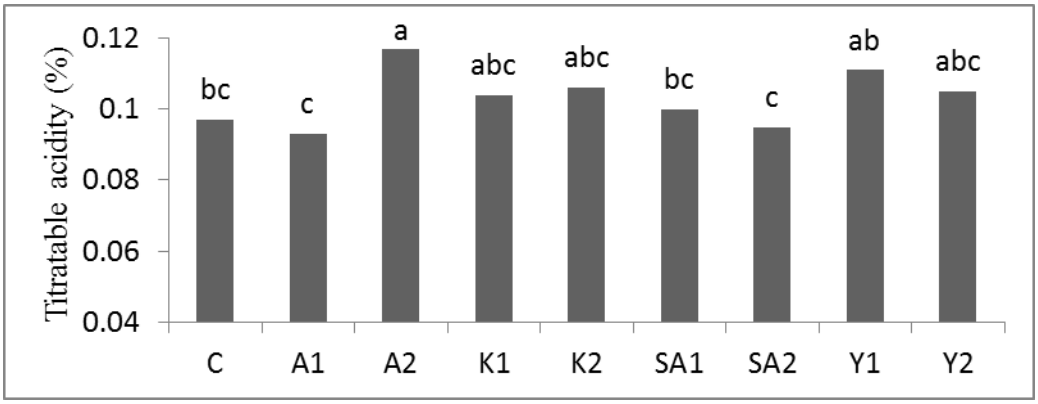
Abscisic acid at higher concentration increased TA of snap bean pods (Fig. 7.9). Kinetin and yeast extract at all concentrations had a comparable amount of TA with ABA at the higher concentration (Fig. 7.9).

In this greenhouse experiment, foliar application of growth regulators and their interaction with drought stress failed to show a consistent and significant effect on length and diameter of pods, TSS, Zn, Fe, Ca, K, P and protein concentrations in snap bean pods (Appendices 20 and 21).

Control SA at 10^{-5} M

SA at 10^{-4} M ABA at 10^{-5} M

Fig. 7.8 The effect of growth regulators on snap bean pods: The control (R1NC), SA at 10^{-5} M (R1NS1), SA at 10^{-4} M (R2NS2) and ABA at 10^{-5} M (R4NA1) concentrations on appearance of snap bean pods under drought stress conditions.



C=Control
A1=10^{-5} M ABA
A2=10^{-4} M ABA
K1=10^{-5} M kinetin
K2=10^{-4} M kinetin
SA1=10^{-5} M Salicylic acid
SA2=10^{-4} M Salicylic acid
Y1=4 g l^{-1} yeast extract
Y2=8 g l^{-1} yeast extract

Fig. 7.9 Pod titratable acidity (%) as a result of growth regulator treatment. The same lower letter on the bars indicates that data are not statistically significant according to the LSD at $P \leq 0.05$

7.5 Discussion

Under unstressed conditions, yeast extract at the higher concentration tended to increase total yield of snap bean under greenhouse conditions although yield was not significantly different to the control. A significant yield increase may be anticipated if yeast extract was applied at earlier growth stages. Under unstressed conditions, ABA and kinetin reduced total yield, pod number and pod dry weight of snap bean pods, particularly at the higher concentration. Under drought stressed conditions, ABA at the lower concentration reduced the impact of drought on pod dry weight.

Quality parameters of snap bean pods under drought were improved by foliar application of growth regulators. Abscisic acid, kinetin and SA improved the marketable yield of snap bean. Involvement of ABA in stress tolerance is well documented. Salicylic acid application maintains membrane integrity during drought stress, enabling the plant to maintain tissue water for continued photosynthesis (Gunes et al., 2007; Farooq et al., 2009b). Kinetin increased proline production in plants under drought (Thomas et al. 1992). Proline reduces the impact of drought stress by stabilizing membrane structure (Ashraf and Foolad, 2007), and is an osmolyte that acts as a reducing agent to generate ATP during recovery from drought (Ashraf and Foolad, 2007). Another report showed that application CK resulted in a greater drought resistance in grasses (Zhang and Ervin, 2004).

In our experiment, SA application at the higher concentration reduced the extent of curving of pods under drought. The reasons of how SA acted to reduce the extent of curving of pods under drought stress needs further investigation. The rough texture and poor appearance of pods under drought stress may be due to poor supply of photo-assimilate from the leaves. Pod curving also caused by unequal tissue turgor in pods, and increased fiber in the pod string. Salicylic acid improved the texture and appearance of snap bean pods under drought stress compared to the control, possibly due to maintenance of tissue water level that withstands the impact of drought (Farooq et al., 2009b). This maintained tissue turgor may have allowed photosynthesis to proceed and overcome some of the deleterious effects of drought that deteriorate pod texture and appearance.

Growth regulators, specifically ABA, kinetin and SA reduced the quality of the pods by degrading the texture and appearance of snap bean pods under unstressed condition. The

negative impact of ABA in plant development was reported by Artlip et al. (1995), Sreenivasulu et al. (2012) and Hayashi et al. (2014). Another report indicated a dramatic increase in CK resulted in necrotic lesions, severe wilting and cell death (Novak et al., 2013), likely associated with the rough pod texture and poor appearance. Salicylic acid also resulted in rougher and poorer pods under unstressed conditions. Although reports on kinetin (Hedin and McCarty, 1994) and SA (Hayat et al., 2010) demonstrate a yield increase in some crops, these growth regulators failed to improve yield and quality of snap bean pods under unstressed conditions in our research.

7.6 Conclusion

Foliar application of growth regulators during flowering did not reduce the impact of drought stress on total pod yield, pod number and pod dry weight of snap bean. However, foliar application of ABA (at lower concentration), kinetin and SA were able to improve the quality of snap bean pods under a short drought stress during flowering leading to improved marketable yield. In contrary, growth regulator application had negative effects when drought was not present. Overall, our result showed that judicious application of SA could reverse the negative impact of drought stress on quality of snap bean pods. The use of yeast extract under unstressed conditions should be further investigated by applying at earlier developmental stages.

CHAPTER 8

8. General discussion, conclusions and future research

Vegetables play a major role in the world economy (Weinberger and Lumpkin, 2007; Wells et al., 2014) and can improve profitability of small-scale farmers (Weinberger and Lumpkin, 2005). Commercial vegetable production including snap bean relies on intensive production systems that demand high level of inputs and advanced infrastructure (Pennsylvania State University, 2014). Regardless of their contribution to poverty alleviation, vegetable production by small-scale resource-limited farmers is still constrained by high input costs (Achterbosch et al., 2007).

Biological N_2 fixation, a key source of N for poor farmers, constitutes one of the potential solutions and may play a key role in sustainable low cost bean production (Chianu et al. 2011). Extensive reports are available on the realization of rhizobium inoculations in increasing yields of chickpea (Bhuiyan et al., 2008), soybean (Sall and Sinclair, 1991), common bean (Bliss, 1993a; Bildirici and Yilmaz, 2005; Otieno et al., 2009). However, the major emphases of previous studies were on the role of N_2 fixation on dry seed yield of mature legume crops, which maximizes the N contribution of this beneficial association. The use of rhizobial inoculant as a source of N for vegetable legume production including snap bean which is harvested early in the season is not well studied. Therefore, one of the general objectives of the thesis was to determine the potential of snap bean production under low input production systems using rhizobium inoculation as N source and under rain fed condition. The N_2 fixation, yield, pod quality and pod nutrient concentrations were assessed under this objective. The results under irrigation experiment were also presented for comparison.

Results showed that applied N improved pod yield and quality of snap bean pods, and applied N improvements were almost always better than those from rhizobium inoculations. However, significant improvements also resulted from rhizobium inoculation compared to the control treatment, particularly under rain fed conditions. While rhizobia inoculation was not as effective as high rates of inorganic N fertilizer, it still remains a viable and potentially less expensive alternative for improving of snap bean yield under rain fed conditions. Most

importantly, snap bean pod yield and quality improvements can be achieved by a N_2 fixation system at earlier harvest of horticultural maturity in snap bean.

According to Singh (2001) high yielding snap bean cultivars adapted to low input production systems are essential for sustainable vegetable production. Melkassa 1 was suited to low input production system due to abundant nodulation, high N_2 fixation and high yield across locations under rain fed conditions. The interaction of N treatment by cultivar showed that rhizobium inoculation improved pod appearance the most, particularly Melkassa cultivars, indicating these cultivars had a compatible rhizobium-host plant interaction. However, cultivars generally had fewer nodules under irrigation, particularly at Hawassa and Ziway. Commercial cultivars also yielded numerically better than Melkassa cultivars under irrigation. In the literature, nodulation and N_2 fixation depend on the genotype of the host plant, rhizobium strain, and their interaction with soil and environmental conditions (Bordeleau and Prevost, 1994). Under optimized environmental conditions, genetically superior genotypes of common bean that are nodulated with efficient rhizobium strain are able to fix enough N_2 to support grain yield (Bliss, 1993a). Nitrogen derived from biological fixation is 50 – 70 % more efficient than applied N because only 30 – 50 % of the latter is recovered by plants (Bliss, 1993b).

Debre Zeit, which is characterized by a tepid to cool sub-moist climate zone, had the most suitable environment for snap bean N_2 fixation. Apart from yield improvement using rhizobium inoculation at all locations, snap bean growers around Debre Zeit can benefit the most from N_2 fixation.

Common bean is an important source of protein (Broughton et al., 2003). Much genetic variability exists in preventing nutritional deficiencies because common bean has high protein and Zn and Fe (Prolla et al., 2010). Common bean grain contains up to 20% protein (Sammàn et al., 1999). For comparison, our results showed that snap bean pods on dry weight basis had comparable protein concentration (16 – 24%) to seed of common bean. The value ranged from 1.3 to 2% on fresh weight basis. Nitrogen concentration in plant tissue is greater at earlier growth stage than at later growth stages (Barker and Pilbeam, 2007).

Snap bean pods are an excellent sources of calcium (Ca) which is highly bioavailable compared to other foods (Quintana et al., 1999a). Snap bean pods have higher Ca concentrations than both pods (Quintana et al., 1999b) and seeds (Peirce, 1987) of common bean. Our

investigations showed that cultivars interacted with location and year to affect Ca concentration. This result confirms a previous report (Quintana et al., 1999b).

Zinc (Zn) concentration in snap bean pods was influenced by growing environment. Our experiment showed that the presence of high Zn concentration in the soil, an important element to prevent nutrient deficiencies in human nutrition, may not necessarily lead to higher pod Zn concentration. Other factors including soil pH (Jeffery and Uren, 1983) should be monitored to increase Zn concentration in snap bean pods. Selecting a suitable environment for higher Zn concentration can be considered to optimize Zn content. Hawassa, which is found in a hot to warm sub-moist humid climate, was suitable for pod protein and Zn concentrations. In summary, environmental variables have a profound effect on productivity, quality and nutrient concentrations of snap bean pods.

In tropical regions of the world, moisture may be too much or too low, and can severely affect crop growth and development. Drought may be short and not cause severe impact on a crop, or it may be long enough to cause physiological destruction or crop death (Acquaah, 2012). Under rain fed conditions, rainfall is sometimes erratic in frequency, quantity and distribution to affect the performance of crops (Acquaah, 2012). Studies also showed that the impact of drought stress varies with genotype and growth stage (Acquaah, 2012).

Drought stress due to insufficient and unpredictable rainfall is a profound constraint in dryland bean production areas (Acosta-Gallegos and Kelly, 2012). Although snap bean is produced under irrigation in eastern Africa, our research ascertained the possibility of producing snap bean under dryland agriculture. Unlike common bean, breeding for drought tolerance or resistance in snap bean is not widely conducted. Therefore, one of our objectives was to assess the relative tolerance of snap bean cultivars to drought stress during different developmental stages under greenhouse conditions. Absence of a significant interaction of cultivar by drought stress for all yield and quality parameters indicated that all cultivars in the experiment were similar in response to drought stress during different developmental stages.

Adverse effects of drought stress on yield reduction are well documented in many crops including common bean, and snap bean response would be expected to behave similarly. However, the effect of drought stress during different developmental stages on snap bean pod physical qualities and nutrient concentrations was not known. Snap bean is a high value export

commodity crop in eastern Africa including Ethiopia. Snap bean physical qualities including pod curving, texture and appearance are of prime importance in international markets. Small defects may result in the rejection of pods for export. Another objective was designed to assess the effect of drought stress during different growth stages on snap bean pod physical qualities and nutrient concentrations. Knowing the most sensitive developmental stage of snap bean for drought stress can also help in designing supplemental irrigation during that stage of development.

Pod quality of snap bean is a combination of appearance and physical condition including well-formed and straight pods, bright in color with a fresh appearance, free of defects, tender and firm (Cantwell and Suslow, 1998). Under greenhouse conditions, drought stress during reproductive stages (R6 and R7) resulted in rough, curved and rippled pods which are poor in appearance. Our investigation suggests that snap bean growers should have the possibility of irrigating their snap bean to supplement the natural rain when and where drought occurs particularly during R6 and R7 developmental stages.

Effects of drought stress on nutrient concentrations were not consistent in different reports. The increased or decreased levels of nutrients in response to drought stress depend on plant species and organ (Nchimbi-Msolla and Tryphone, 2010; Sharma and Dubey, 2011). Our investigation indicated that total protein and Zn concentrations showed progressive increases from unstressed to drought stressed during V4.4 and reproductive stages (R6 and R7) but Fe concentration was reduced. P and K concentrations were increased when drought occurred at R6 stage. However, the increase in nutrient concentration is at the expense of yield. Protein content per plant was decreased by 25, 30 and 24% due to drought stress at V4.4, R6 and R7 developmental stages, respectively. Zinc content also decreased by 25, 27 and 21% due to drought stress at V4.4, R6 and R7 developmental stages, respectively. Therefore, drought stress decreased the quality of snap bean pod in terms of nutrient content per unit area.

Drought is a common phenomenon in tropical regions particularly in sub-Saharan African countries including Ethiopia. Strategies are required at least to minimize the impact of drought stress on crops grown in drought prone areas. Our last objective was to test foliar application of growth regulator to reduce the impact of drought stress occurred during R6 on snap bean yield and pod qualities.

Foliar application of SA under greenhouse conditions showed very promising results in reversing the impact of drought stress particularly on pod quality parameters including marketable yield, pod curving, texture and appearance of snap bean pods. Salicylic acid is a monohydroxybenzoic acid, a type of phenolic acid, and a beta hydroxy acid. This colorless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone. Reports showed the involvement of SA in plant drought responses (Hayat et al., 2010; Hayat et al., 2012; Habibi, 2012; Sadeghipour and Aghaei, 2012). Moreover, SA is more economical to use in mitigating drought stress (Ullah et al., 2012). However, foliar applications of most of the growth regulators including SA had a negative impact on pod quality parameters under unstressed conditions. In contrast, yeast extract tended to show a positive impact under unstressed conditions. Despite reports on kinetin (Hedin and McCarty, 1994) and SA (Hayat et al., 2010) in increasing yields of many crops, these growth regulators failed to improve yield and quality of snap bean pods under unstressed conditions in our investigation.

Concluding remarks:

- Rhizobial inoculation and applied inorganic N increased the yield of snap bean pods under rain fed conditions by 18 % and 42 %, respectively.
- Pod yield improvement can be achieved by N_2 fixation system sustained until the commercial maturity of snap bean pods, and viable snap bean production can be realized using rhizobial inoculant under rain fed conditions.
- Melkassa 1 was the most suitable cultivar for a low cost production system due to its successful nodulation character and the greatest N_2 fixation levels under rain fed conditions.
- Conditions at Debre Zeit were the most conducive for supporting biological N_2 fixation for snap bean production under rain fed conditions.
- Commercial cultivars possess best pod quality characteristics such as texture and appearance, and their yield performances were better under irrigation.
- Cultivars interacted with locations to affect pod traits including TSS and concentrations of protein, Ca, and K under rain fed conditions.

- Snap bean pods produced at Debre Zeit and Hawassa were similar in several traits but Zn concentration was highest at Hawassa under rain fed conditions. Ziway, with a more arid climate and soil pH above 8.0, was the least favorable location for snap bean production.
- Short drought stress during reproductive stages (R6 and R7) under greenhouse conditions resulted in very rough, curved and rippled pods which are poor in appearance.
- Short drought stress under greenhouse conditions increased protein, P and Zn concentrations; however, it reduced Fe concentration in snap bean pods. Additionally, drought stress during flowering stage (R6) increased P and K concentrations.
- Foliar application of SA under greenhouse conditions showed very promising results in reversing the impact of drought stress particularly on pod quality parameters including marketable yield, pod curving, texture and appearance of snap bean pods.

Suggested future research related to this project includes the following:

1. The complete absence of nodulation and N_2 fixation in snap bean in most of plots at Hawassa and Ziway during the dry season under irrigation requires further investigation.
2. Breeding research is needed to improve the pod texture and appearance of cultivar Melkassa 1 to gain benefits from its greatest N_2 fixation and high yielding potential.
3. The reasons behind increasing and decreasing nutrient concentrations in snap bean pods in response to drought stress need investigation (particularly protein, Zn, iron, Ca, P and K).

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APPENDICES

Appendix 1. Average rainfall, maximum and minimum temperature during 2011 and 2012 growing seasons at Debre Zeit, Hawassa and Ziway, Ethiopia. Ten year normal climate, altitude and climate zone of each location†

		Debre Zeit			Hawassa			Ziway		
Year		Rainfall	Max. T‡	Min. T§	Rainfall	Max. T‡	Min. T§	Rainfall	Max.T‡	Min. T§
		mm	^{o}C	^{o}C	mm	^{o}C	^{o}C	mm	^{o}C	^{o}C
	July	134.6	26.9	13.5	129.6	25.7	12.8	133.7	25.8	14.8
2011	August	241.7	25.0	14.9	157.3	25.3	13.0	114.8	24.6	15.1
	September	82.6	25.0	14.9	113.3	25.7	13.3	56.2	25.5	13.3
	Annual	724.1	26.4	11.3	776.1	28.0	12.1	598.3	29.1	13.0
	February	0.0	28.1	7.6	3.4	31	9.5	1.1	30.7	10.0
2012	March	26.2	29.1	10.0	13.1	30.9	11.7	2.9	29.8	15.2
	April	53.8	27.6	13.3	149.6	30.7	14.3	83.2	32.5	12.5
	July	197.4	25.0	13.5	232.5	24.9	14.7	326.3	23.2	15.0
2012	August	256.5	24.5	12.6	72.7	24.4	14.5	171.4	24.3	14.7
	September	103.0	25.6	12.5	139.8	27	15.3	136.6	27.8	9.7
	Annual	726.3	26.7	10.4	884.8	28.1	12.7	856.8	28.6	12.4
10 years	Annual Aver.	747.0	26.4	10.7	786.5	27.9	12.3	763.9	27.5	13.9
Altitude	m above sea level	1950			1700			1645		
Climate Zone¶		Tepid to cool sub-moist			Hot to warm sub-moist humid			Tepid to cool semi-arid		

†Data collected by Debre Zeit Agricultural Research Center (Debre Zeit), South Agricultural research Center (Hawassa), and Adame Tulu Agricultural Research Center (Ziway), ‡Maximum Temperature, §Minimum Temperature, ¶According to Ministry of Agriculture (2000) in Ethiopia

Appendix 2. Soil physicochemical characteristics at Debre Zeit, Hawassa and Ziway, Ethiopia during the 2011 and 2012 growing seasons

Profile Code	Debre Zeit 2011	Hawassa 2011	Ziway 2011	Debre Zeit 2012	Hawassa§§ 2012	Ziway 2012
Sand (%)	13.59	47.03	83.62	15.57	51.69	74.17
Silt (%)	14.75	29.66	14.33	10.30	30.20	17.22
Clay (%)	71.65	23.31	2.05	74.14	18.12	8.61
Texture class†	clay	loam	sandy loam	clay	loam	sandy loam
pH-H2O (1:2.5) ‡	6.98	6.10	8.38	6.98	6.10	8.20
pH-KCl (1:2.5) ‡	5.96	5.31	7.61	6.02	5.22	7.58
EC (ms cm^{-1}) (1:2.5)	0.16	0.17	0.15	0.26	0.17	0.26
Exch.Na (cmolc kg^{-1} soil) §	0.44	0.65	1.19	0.70	0.60	1.35
Exch.K (cmolc kg^{-1} soil) §	0.36	1.50	1.84	0.32	2.41	2.20
Exch.Ca (cmolc kg^{-1} soil) §	32.32	12.93	18.58	28.28	12.93	21.82
Exch.Mg (cmolc kg^{-1} soil)§	15.35	11.31	6.87	12.12	8.08	0.81
sum of cations (cmolc kg^{-1} soil)	52.70	36.01	34.69	44.35	36.01	37.77
CEC (cmolc kg^{-1} soil)	48.47	26.39	28.48	41.42	24.01	26.18
Organic Carbon (%) ¶	1.5	1.59	0.96	1.47	1.55	1.15
Nitrogen (%) ††	0.11	0.11	0.10	0.08	0.10	0.07
Available P (mg P_2O_5 kg^{-1} soil) #	43.66	49.32	43.81	41.89	91.68	83.46
Available K (mg K_2O kg^{-1} soil) §	158.22	620.7	778.91	141.18	973.64	864.11
$CaCO_3$ (%)						
Exchangeable sodium % (ESP) §	0.83	1.80	3.42	1.58	1.66	3.58
Micronutrients ‡‡						
Cu (mg kg^{-1} soil)	2.04	0.30	0.33	1.47	0.39	0.32
Fe (mg kg^{-1} soil)	12.46	28.96	3.13	10.64	25.93	4.58
Mn (mg kg^{-1} soil)	9.27	20.76	2.70	7.82	27.03	4.63
Zn (mg kg^{-1} soil)	0.86	3.61	1.08	0.86	3.78	1.50

Methods: †Hydrometer; ‡Acid neutralization; §Ammonium acetate; #Olsen; ¶Walkley and Black; ††Kjeldahl; ‡‡ Instrumental, §§The same soil from Hawassa was used for the greenhouse experiment Chapter 6

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Appendix 3. Descriptions of snap bean cultivars used in the experiments.

Cultivar	Seed Source	Accession No. /pedigree	Plant size	Pod type/size	Flower colour
Andante	Pop Vriend Seeds	Proprietary information	Small	Very fine	White
Boston	Pop Vriend Seeds	Proprietary information	Medium	Fine	White
Contender Blue	Holmes	Proprietary information	Medium	Medium fine	White
Lomami	Pop Vriend Seeds	Proprietary information	Medium	Fine	White
Melkassa 1	CIAT (MARC)*	L-12	Large	Bobby/Large	White
Melkassa 3	CIAT (MARC)*	BC-4.4	Large	Bobby/Large	Purple
Paulista	Seminis	Proprietary information	Medium	Fine	White
Volta	Pop Vriend Seeds	Proprietary information	Medium	Fine	White

*Melkassa Agricultural Research Center, Ethiopia

Appendix 4. *P*-values from mixed model ANOVA F-test for total yield, number of pods per plant, pod dry weight per plant, nodule number per plant, nodule dry weight per plant, average nodule diameter, days to flowering, days to maturity, plant height, leaf area index, and harvest index of snap bean affected by nitrogen treatment, cultivar and location in 2011 and 2012 under rain fed conditions

	Pod total yield	Number of pods plant^{-1}	Pod dry weight plant^{-1}	Nnodule number plant^{-1}†	Nodule dry weight plant^{-1}†	Nodule diameter	Days to flower	Mays to maturity	Plant height	Leaf area index	Harvest index
Source	t ha^{-1}		g		mg	mm			cm		
Nitrogen											
Treatment (N)	0.0001***	0.0436*	0.0387*	0.0317*	0.0025**	0.0001***	0.0477*	0.0238*	0.2967	0.0467*	0.0001***
Cultivar (V)	0.0068**	0.0183*	<.0001***	0.0044**	0.0019**	0.0001***	0.0025**	<.0001***	0.0154*	0.0005***	0.1304
Location (L)	0.0212*	0.0118*	0.0202*	0.7464	0.267	0.0488*	0.0722	0.0046	0.4827	0.202	0.0205*
L*V	0.3933	0.06	0.1122	0.0789	0.303	0.3688	0.3041	0.0467	0.2126	0.0548	0.0001***
L*N	0.2128	<.0001***	0.1434	0.7114	0.599	0.0968	0.0261*	0.3374	0.6806	0.2227	0.0001***
V*N	0.6449	0.1252	0.4943	0.3372	0.146	0.9302	0.8023	0.1519	0.3455	0.8424	0.5145
L*V*N	0.9216	0.9868	0.7237	0.2967	0.679	0.8207	0.3806	0.9038	0.9711	0.9657	0.4863
Year*L*V	0.2194	0.086	0.228	0.2031	0.1005	0.2829	0.0119*	0.0031**	-	0.3182	-

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

† Data were log10 transformed for analysis.

Appendix 5. *P*-values from mixed model ANOVA F-test for marketable yield, pod length, pod diameter, pod curvature, pod texture, pod appearance, titratable acidity (TA) and total soluble solids (TSS) of snap bean affected by nitrogen treatment, cultivar and location in 2011 and 2012 under rain fed conditions

	Marketable yield	Pod length	Pod diameter	Pod curvature	Texture	Appearance	TA	TSS
Source	t ha^{-1}	mm	mm		1-5	1-5	%	^{o}Brix
Nitrogen Treatment (N)	0.0001***	0.3794	0.1192	0.8464	0.0986	0.0054**	0.002**	0.0727
Cultivar (V)	<.0001***	<.0001***	<.0001***	0.5088	0.0316*	0.0046**	0.0072**	0.0225*
Location (L)	0.0173*	0.0868	0.3865	0.5525	0.6253	0.6626	0.0016**	0.5567
L*V	0.473	0.3774	0.292	0.3277	0.272	0.5378	0.2464	0.0001***
L*N	0.1543	0.2169	0.9079	0.3826	0.0078**	0.9636	0.1993	0.8423
V*N	0.6966	0.2525	0.8376	0.7694	0.0299*	0.0226*	0.6749	0.4801
L*V*N	0.7419	0.4971	0.8746	0.4776	0.6752	0.6962	0.0567	0.8689

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 6. *P*-values from mixed model ANOVA F-test for protein, phosphorus (P), zinc Zn), iron (Fe), calcium (Ca) and potassium (K) concentration of snap bean pods affected by nitrogen treatment, cultivar and location in 2011 and 2012 under rain fed conditions

	Protein concentration	Protein content	P	Zn	Fe	Ca	K
Source	%	(Kg ha^{-1})	%	ppm	ppm	%	%
Nitrogen Treatment (N)	0.3093	0.0395*	0.0232*	0.3792	0.3837	0.4652	0.1106
Cultivar (V)	0.050	0.0188*	0.0131*	0.0132*	0.1563	0.0178*	0.069
Location (L)	0.9413	<.0001***	0.8254	0.001**	0.1025	0.0753	0.1482
L*V	0.8561	0.9546	0.6891	0.1245	0.0552	0.9281	0.0051**
L*N	0.8768	<.0001***	0.6424	0.2873	0.1170	0.1451	0.0197*
V*N	0.641	0.3399	0.162	0.5638	0.4004	0.7692	0.8836
L*V*N	0.6985	0.3648	0.6057	0.5623	0.4709	0.3658	0.693
Year*L*V	0.0469*	0.003**	-	-	-	0.0136*	-

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 7. Days to flowering snap bean affected by cultivar by location interaction at in 2011 and 2012 under rain fed conditions

Cultivar	2011			2012		
	Location			location		
	Days to flowering			Days to flowering		
	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	41.2ij	40.3kl	39.9l	42.1ij	42.6hi	39.6l
Boston	45.3fg	47.3a	45.2cd	44.7ab	43.8cde	44.7ab
Contender Blue	41.9j	44.4d	41.6hi	43.1e-h	43.1fgh	41.6j
Lomami	42.3i	43.2e	41.6hi	43.6def	43.3efg	41.6j
Melkassa 1	46.1fgh	47.2a	44.8cd	45.2a	44.1bcd	44.6ab
Melkassa 3	42.9j	43.3e	39.8l	43.6def	43.2efg	39.9l
Paulista	41.6hi	43.1ef	42.1gh	44.2bc	43.3efg	42.1ij
Volta	41.1ghi	43.0fef	40.jkl	42.9gh	43.3efg	40.7k

Means followed by the different letters in an interactions grouping column differ significantly based on LSD, $P \leq 0.05$

Appendix 8. Days to maturity of snap bean affected by cultivar by location interaction at in 2011 and 2012 under rain fed conditions

Cultivar	2011			2012		
	Location			Location		
	Days to maturity			Days to maturity		
	Debre Zeit	Hawassa	Ziway	Debre Zeit	Hawassa	Ziway
Andante	64.6ef	61.3j	59.6k	63.2jkl	63.8ijk	60.1p
Boston	70.1b	68.3c	64.0fg	69.4b	67.2d	65.4e
Contender Blue	66.0d	65.4de	61.3j	64.1ghi	64.4ghi	62.6lm
Lomami	66.0d	64.2fg	62.6i	66.6d	65.3e	62.3mn
Melkassa 1	71.7a	68.2c	63.8fgh	70.6a	68.2c	65.6e
Melkassa 3	64.2fg	64.3f	61.1j	63.9hij	64.6fgh	60.9o
Paulista	64.3ef	64.1fgh	63.0hi	65.7e	65.2ef	63.0klm
Volta	64.6ef	64.0fgh	63.1ghi	64.2ghi	64.9efg	61.7n

Means followed by the different letters in an interactions grouping column differ significantly based on LSD, $P \leq 0.05$

Appendix 9. Plant height and leaf area index of snap bean as affected by cultivar and nitrogen treatment under rain fed conditions in 2011 and 2012

	Plant height	Leaf area index
	cm	
Nitrogen treatment		
0 kg ha^{-1}	31.4	1.3b
Rhizobium etli (HB 429)	33.9	1.5ab
100 kg ha^{-1}	37.4	1.9a
Cultivar		
Andante	25.2b	0.9c
Boston	32.8ab	1.2b
Contender Blue	30.8ab	1.4b
Lomami	33.6ab	1.5b
Melkassa 1	40.1a	2.1a
Melkassa 3	39.5a	2.0a
Paulista	33.8ab	1.4b
Volta	38.0a	1.5b

Means followed by the different letters in an interactions grouping column differ significantly based on LSD, $P \leq 0.05$

Appendix 10. Harvest index of snap bean as influenced by interactions of nitrogen treatment by location and cultivar by location under rain fed conditions in 2012

	Harvest index		
	Debre Zeit	Hawassa	Ziway
Nitrogen treatment			
0 kg ha^{-1}	0.452ab	0.330e	0.361de
Rhizobium etli (HB 429)	0.463a	0.451ab	0.382cd
100 kg ha^{-1}	0.434ab	0.448ab	0.411bc
Cultivar			
Andante	0.468abc	0.448a-d	0.316h
Boston	0.452a-d	0.451a-d	0.327gh
Contender Blue	0.447a-d	0.426a-d	0.389d-g
Lomami	0.437a-d	0.406b-e	0.404b-e
Melkassa 1	0.474a	0.396def	0.449a-d
Melkassa 3	0.407cde	0.360e-h	0.396def
Paulista	0.470ab	0.360e-h	0.464abc
Volta	0.440a-d	0.430a-d	0.335fgh

Means followed by the different letters in an interactions grouping column differ significantly based on LSD, $P \leq 0.05$. Letters a-g indicate all alphabetical letters included in the range from a to g

Appendix 11. *P*-values from mixed model ANOVA F-test for marketable yield, pod length, pod diameter, pod curvature, pod texture, pod appearance, titratable acidity (TA) and total soluble solids (TSS) of snap bean affected by nitrogen treatment, cultivar and location in 2012 under irrigated conditions

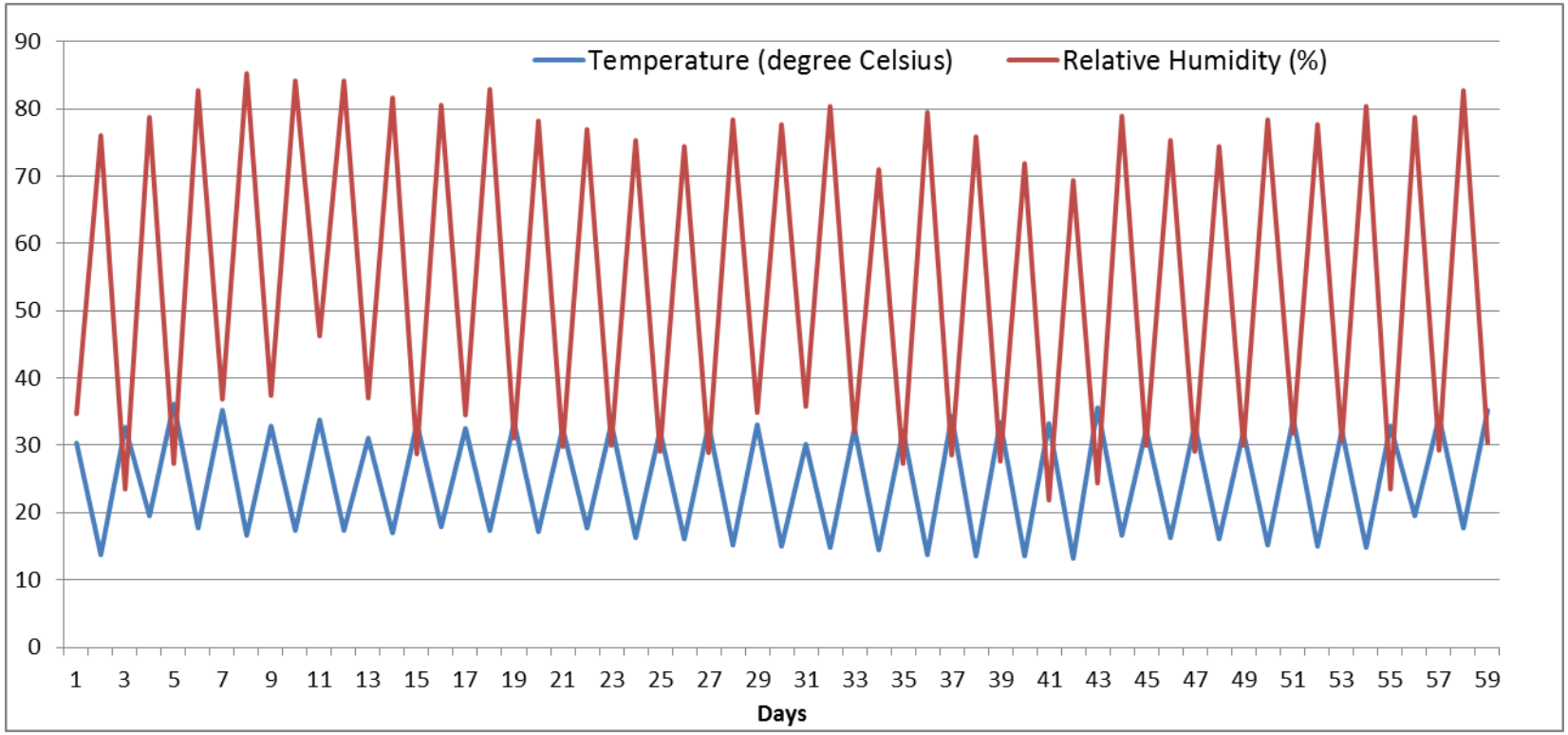
	Marketable yield	Pod length	Pod diameter	Pod curvature	Texture	Appearance	TA	TSS
Source	(t ha^{-1})	mm	mm		1-5	1-5	%	^{o}Brix
Nitrogen Treatment (N)	0.0007***	0.0073**	0.8922	0.7514	<.0001**	<.0001***	<.0001***	<.0001***
Cultivar (V)	<.0001***	<.0001***	<.0001***	0.2165	<.0001**	<.0001***	0.1168	0.3701
Location (L)	<.0001***	0.0179*	0.059	0.1134	0.1918	0.0823	0.0506	0.3901
L*V	0.0012**	0.0074**	0.0097**	0.7915	0.0036**	0.0003***	0.0047**	0.0005***
L*N	0.1828	0.0002***	0.6514	0.4707	0.0059**	<.0001***	0.1271	<.0001***
V*N	0.995	0.0944	0.6181	0.5158	0.0044**	0.0021**	0.5221	0.9308
L*V*N	0.9918	0.05	0.756	0.7911	0.6493	0.0697	0.8575	0.4561

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 12. *P*-values from mixed model ANOVA F-test for protein, phosphorus (P), zinc Zn), iron (Fe), calcium (Ca) and potassium (K) concentration of snap bean pods affected by nitrogen treatment, cultivar and location in 2011 and 2012 under irrigated conditions

	Protein	P	Zn	Fe	Ca	K
Source	%	%	ppm	ppm	%	%
Nitrogen						
Treatment (N)	0.0046**	0.3196	0.6749	0.4883	0.6352	0.5418
Cultivar (V)	<.0001***	0.0021**	0.0956	0.7636	<.0001***	0.0001***
Location (L)	0.0004***	0.0013**	0.0004***	0.1748	0.0027**	0.0002***
L*V	<.0001***	<.0001***	0.1003	0.6525	0.0455*	<.0001***
L*N	0.8914	0.8734	0.5209	0.2505	0.3866	0.2958
V*N	0.5614	0.8748	0.2882	0.4348	0.8037	0.2825
L*V*N	0.1413	0.7015	0.1667	0.4063	0.6128	0.1340

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.



Appendix 13. Daily maximum and minimum temperature and relative humidity in the greenhouse during the growth period from planting until end of harvest. The average of two different experiment times and two greenhouse rooms at each time for drought stress during different developmental stages experiment (September to November in 2012; and October to December in 2013)

Appendix 14. *P*-values from mixed model ANOVA F-test for total yield per plant, pod number per plant, pod dry weight per plant and days to maturity of snap bean affected by drought stress and cultivar under greenhouse conditions

	Total yield plant^{-1}	Number of pods plant-1	Pod dry weight plant-1	Days to maturity
Source	g		g plant^{-1}	day
Drought stress (D)	<.0001***	<.0001***	<.0001***	<.0001***
Cultivar (V)	0.0046**	<.0001***	0.2478	<.0001***
D x V	0.5093	0.4924	0.9902	0.4213

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 15. *P*-values from mixed model ANOVA F-test for for marketable yield per plant, pod length, pod diameter, pod texture, pod appearance, pod curvature, titratable acidity (TA) and total soluble solids (TSS) of snap bean affected by drought stress and cultivar under greenhouse conditions

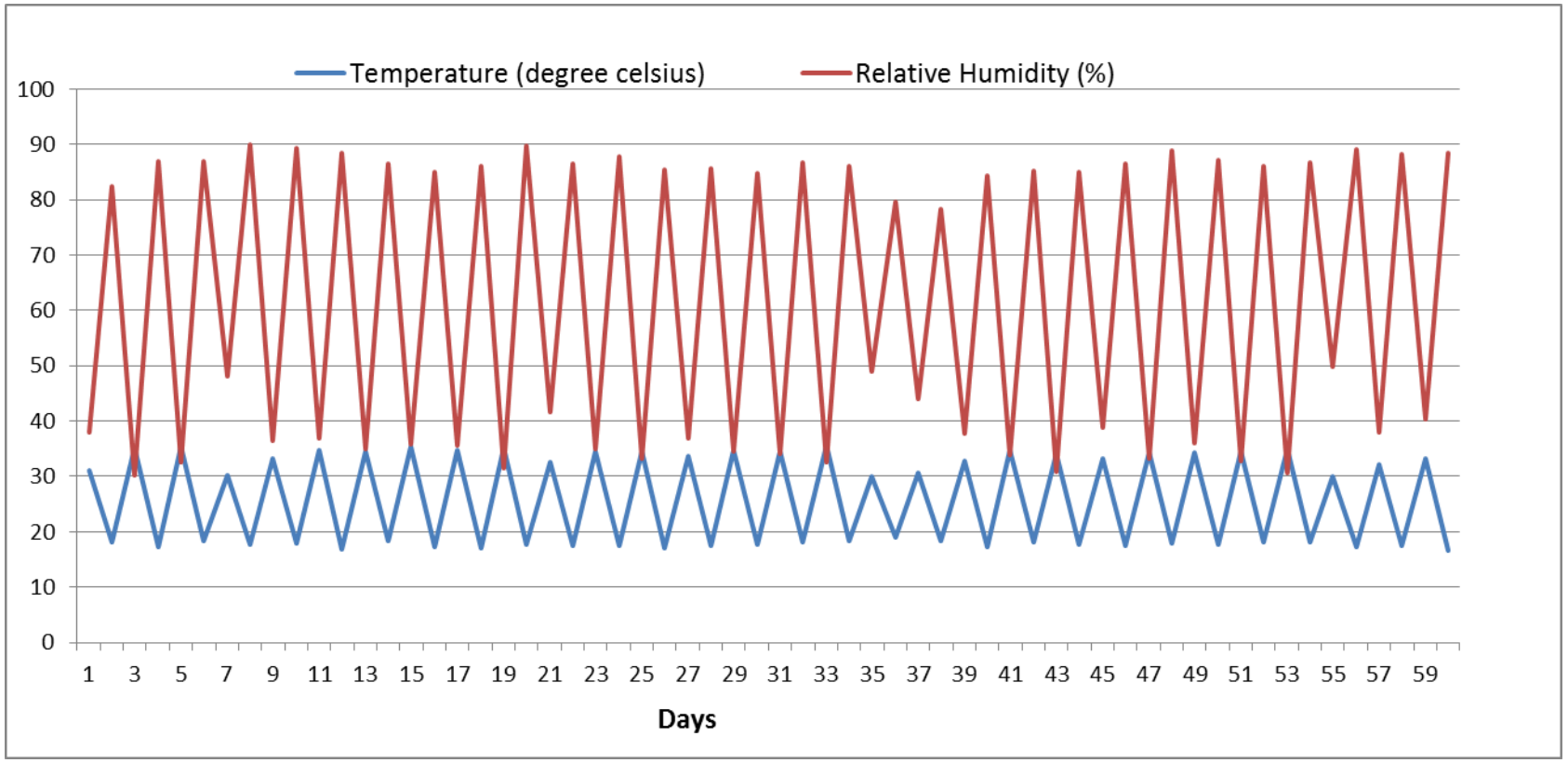
	Marketable yield	Pod length	Pod diameter	Texture	Appearance	Pod curvature	TA	TSS
Source	g plant^{-1}	mm	mm	1-5	1-5		%	^{o}Brix
Drought stress (D)	<.0001***	<.0001***	0.0451*	<.0001***	<.0001***	<.0001***	0.824	0.6269
Cultivar (V)	0.0312*	<.0001***	<.0001***	0.4158	<.0001***	<.0001***	0.5446	0.8091
D x V	0.8504	0.8042	0.5091	0.4984	0.4609	0.1705	0.7203	0.8826

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 16. *P*-values from mixed model ANOVA F-test for protein, phosphorus (P), zinc (Zn), iron (Fe), calcium (Ca) and potassium (K) concentrations of snap bean pods affected by drought stress and cultivar under greenhouse conditions

	Protein	P	Zn	Fe	Ca	K
Source	%	%	ppm	ppm	%	%
Drought stress (D)	<.0001***	0.0481*	<.0001***	<.0001***	0.0037**	<.0001***
Cultivar (V)	0.0334*	0.8793	0.0178*	<.0001***	<.0001***	0.0571
D x V	0.9323	1	0.5935	0.232	0.968	0.9236

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.



Appendix 17. Daily maximum and minimum temperature and relative humidity in the greenhouse during the growth period from planting until end of harvest both first and second experiments of chapter 7.

Appendix 18. Physicochemical characteristics of the soils used in the greenhouse (both first and second experiments of chapter 7)

Profile Code	Hawassa 2014
Sand (%)	47.88
Silt (%)	30.66
Clay (%)	21.46
Texture class†	loam
pH-H2O (1:2.5) ‡	6.66
pH-KCl (1:2.5) ‡	5.74
EC (ms cm^{-1}) (1:2.5)	0.15
Exch.Na (cmolc kg^{-1} soil) §	0.98
Exch.K (cmolc kg^{-1} soil) §	0.86
Exch.Ca (cmolc kg^{-1} soil) §	13.06
Exch.Mg (cmolc kg^{-1} soil)§	4.49
sum of cations (cmolc kg^{-1} soil)	19.39
CEC (cmolc kg^{-1} soil)	25.28
Organic Carbon (%) ¶	1.94
Nitrogen (%) ††	0.21
Available P (mg P_2O_5 kg^{-1} soil) #	77.05
Available K (mg K_2O kg^{-1} soil) §	340.0
$CaCO_3$ (%)	
Exchangeable sodium % (ESP) §	3.86
Micronutrients ‡‡	
Cu (mg kg^{-1} soil)	0.29
Fe (mg kg^{-1} soil)	29.00
Mn (mg kg^{-1} soil)	21.11
Zn (mg kg^{-1} soil)	3.51

Methods: †Hydrometer; ‡Acid neutralization; §Ammonium acetate; #Olsen; ¶Walklay and Black; ††Kjeldahl; ‡‡ Instrumental

Appendix 19. *P*-values from mixed model ANOVA F-test for total yield per plant, pod number per plant, pod dry weight per plant of snap bean affected by drought stress and growth regulator

	Total yield plant^{-1}	Number of pods plant^{-1}	Pod dry weight plant^{-1}
Source	g		g
Drought stress (D)	<.0001***	<.0001***	<.0001***
Growth regulator (GR)	0.1323	0.2597	0.1114
D x GR	0.0005***	0.0221*	0.0018**

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

Appendix 20. *P*-values from mixed model ANOVA F-test for for marketable yield per plant, pod length, pod diameter, pod curvature, pod texture, pod appearance, titratable acidity (TA) and total soluble solids (TSS) of snap bean affected by drought stress and growth regulator under greenhouse conditions

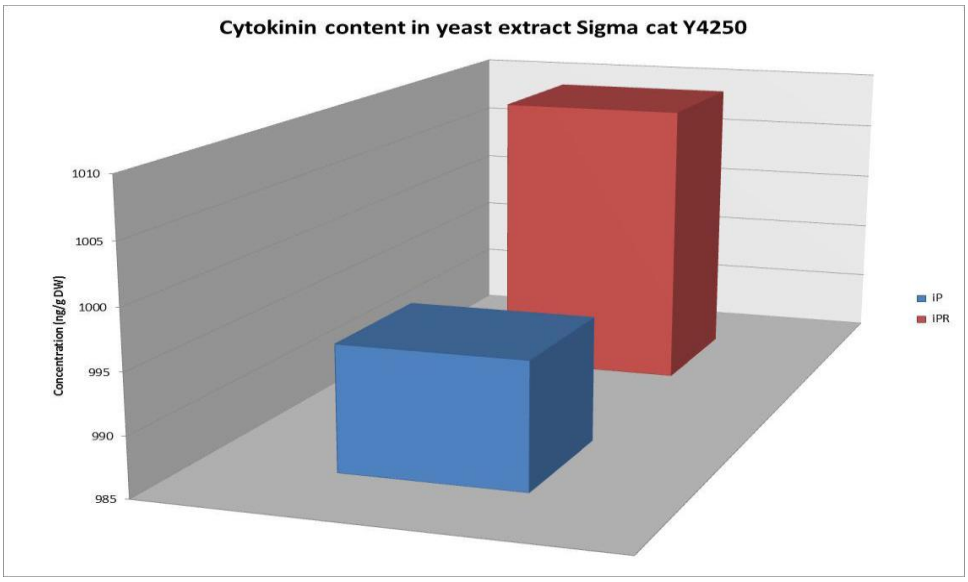
	Marketable yield	Pod length	Pod diameter	Texture	Appearance	Pod curvature	TA	TSS
Source	g plant^{-1}	mm	mm	1-5	1-5		%	^{o}Brix
Drought stress (D)	<.0001***	0.0003***	0.1208	<.0001***	<.0001***	<.0001***	0.0002***	0.3626
Growth regulator (GR)	0.0363*	0.4056	0.7863	<.0001***	<.0001***	0.1602	0.0271*	0.1737
D x GR	<.0001***	0.2637	0.9183	<.0001***	<.0001***	0.0089**	0.2758	0.0835

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.

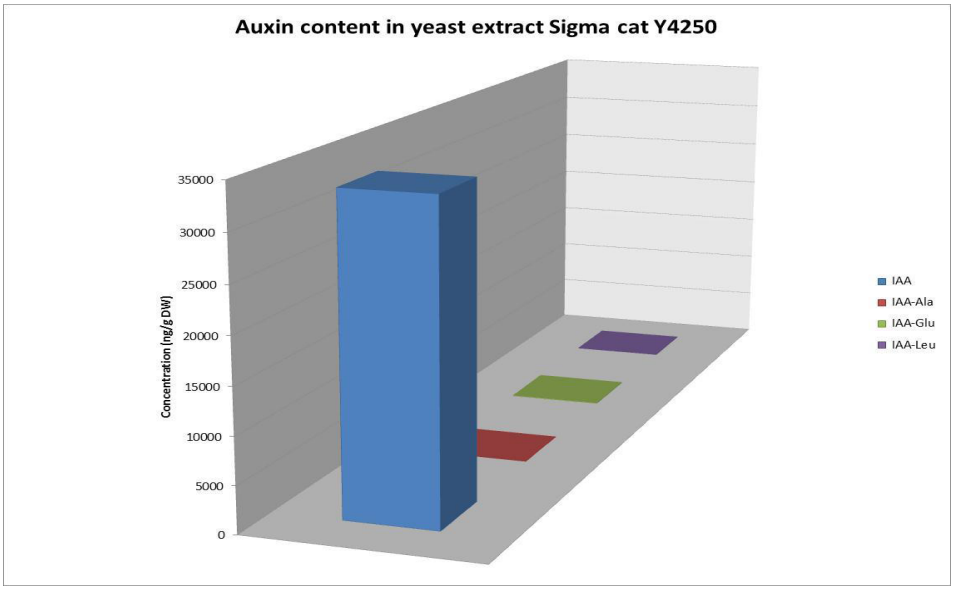
Appendix 21. ANOVA (P values) for protein, phosphorus (P), zinc (Zn), iron (Fe), calcium (Ca) and potassium (K) concentrations of snap bean pods affected by drought stress and growth regulator under greenhouse conditions

Source	Protein	P	Zn	Fe	Ca	K
	%	%	ppm	ppm	%	%
Drought stress (D)	0.0036**	0.0364*	0.0156*	0.4281	0.0004***	<.0001***
Growth regulator (GR)	0.906	0.9985	0.9964	0.9377	0.7493	0.9061
D x GR	0.9124	0.9975	1.000	0.8335	0.8509	0.9932

*,**,***, denote significant at the 0.05,0.01,0.001 probability levels respectively.



Appendix 22. Cytokinin concentrations in yeast extract quantified by National Research Council of Canada, using Lulsdorf et al. (2013) method



Appendix 23. Auxin concentrations in yeast extract quantified by National Research Council of Canada, using Lulsdorf et al. (2013) method