



# **CHAPTER ONE**

## **GENERAL INTRODUCTION**

## **1.1 Importance of grain legumes**

### **1.1.1 Production of common bean and soybean**

Common bean production in Africa is estimated to represent 3,741,000 ha with about 25% of the total world production (11-12 million tons) (FAO, 2006). Figure 1.1 illustrates the areas of bean production in Sub-Saharan Africa (Wortmann et al., 1998). However, due to the development of new bean varieties and increased demand, the hectareage is expected to continue to increase. For example in Ethiopia, one of the biggest bean producers in Africa, bean production is currently extending to various regions of the country, becoming the second most important grain legume with production on 280,000 ha in the 2008/9 cropping year as shown in Figures 1.2 and 1.3 (CSA, 2010; Negash et al., 2011). Due to increasing demand and increased interventions in research and development in legume growing countries, the production of legumes has shown a remarkable increase in both hectareage and in production (Abate, 2012). For instance, in bean producing countries in Africa (e.g., in Eastern African countries such as Tanzania, Kenya and Ethiopia) the production of bean has reached up to 482, 390 and 267 thousand metric tons in the 2010 cropping year (TL II conference, 2011 country report, unpublished). This shows the increased demand and importance for beans and the need to solve production constraints, such as drought, low productivity and reduced profitability for the growers especially for moisture stress areas.

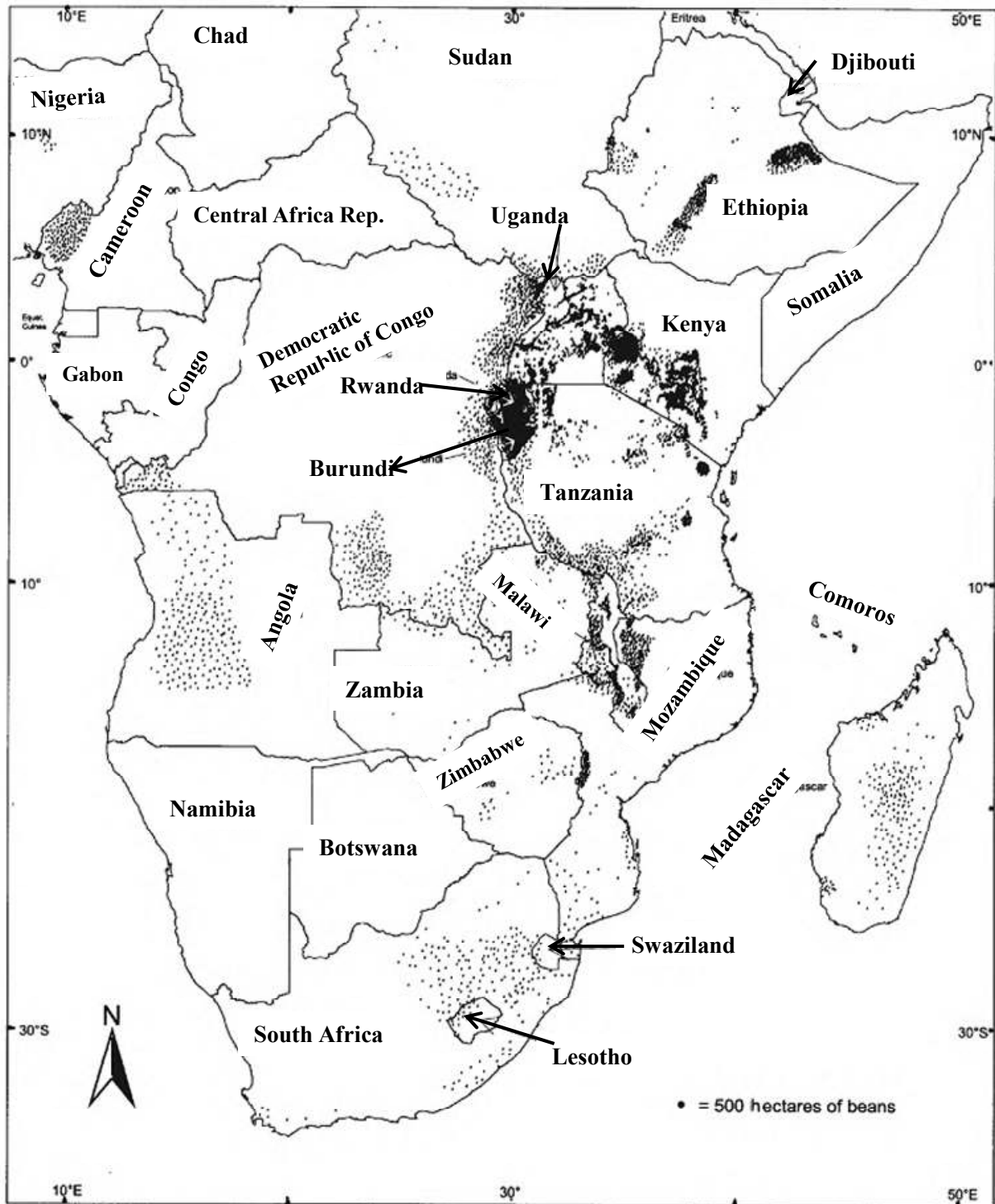
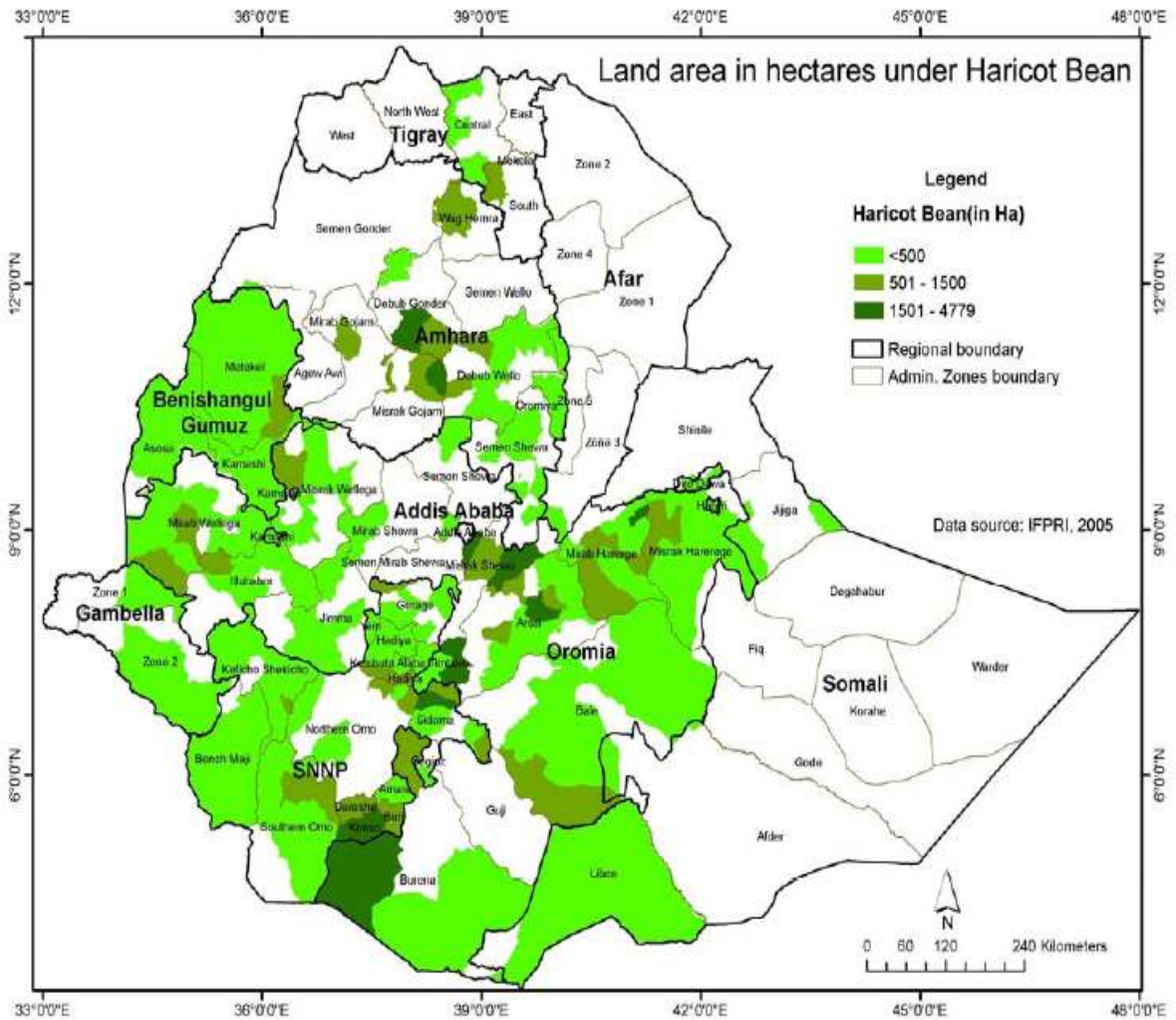
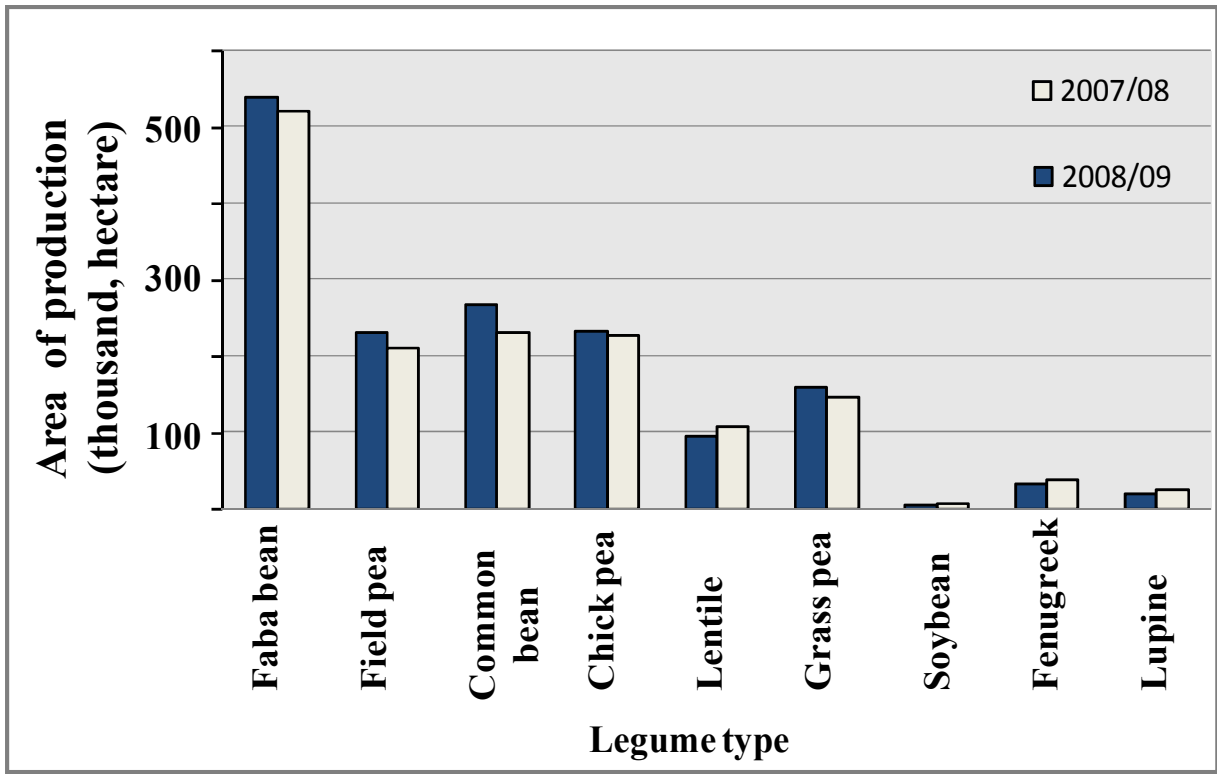


Figure 1.1: Distribution of bean production in sub-Saharan Africa (Source: Wortmann et al., 1998).



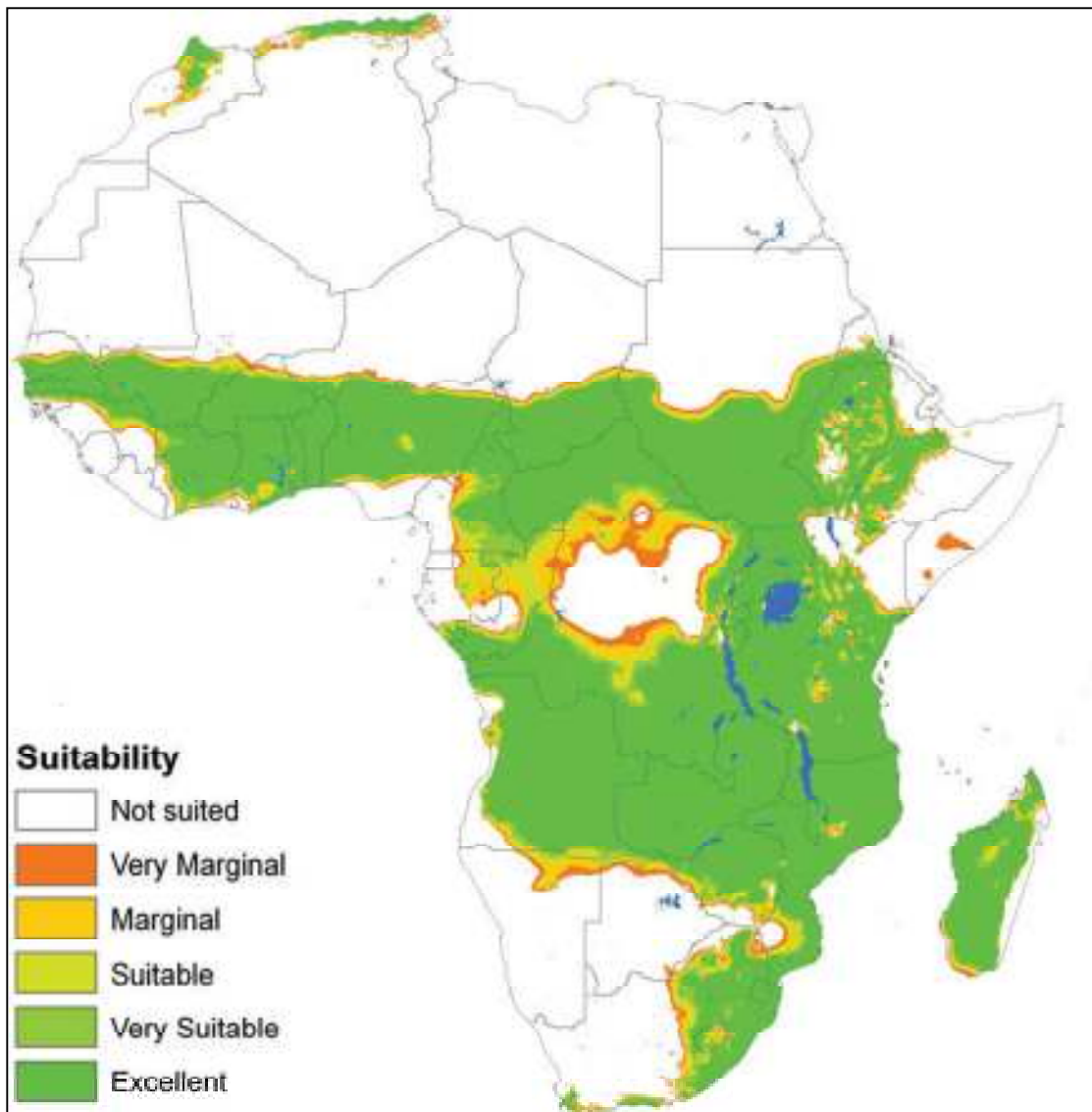
**Figure 1.2:** Geographic distribution of common bean production in Ethiopia for the year 2005

(Source: Negash et al., 2011).



**Figure 1.3:** Estimated area of production of different legumes in Ethiopia for 2007/08 and 2008/09 cropping year (Source: CSA, 2010).

Soybean is produced globally on 94 million hectares of land with production of 223 million tons in 2006/08. Africa only produced 1.5 million tons which is about 1% of the world production (FAOSTAT, 2010). The total area of soybean cultivation in Africa is about 1.3 million ha with the three major soybean producers, Nigeria (625,667 ha), South Africa (199,323 ha) and Uganda (146,667 ha) (FAOSTAT, 2010). This accounts for about 80% of soybean production in Africa. Although the suitability map for soybean production in Africa shows a huge potential for the crop (Figure 1.4), and Africa reports an annual growth rate in soybean production of about 5%, this production increase is still not fast enough. If production growth continues according to this trend, the world-wide production will be 293 million tons in 2020 but with Africa still having a deficit of 196,000t (Abate, 2012). This is in spite of the growing demand for soybean for domestic processing of soybean meal and soybean oil in Africa. Particularly in Ethiopia soybean production has the potential to grow in most parts of the country and the production area coverage is expected to be 6826 ha (Tefera, 2011). This would rank Ethiopia as the 12<sup>th</sup> largest producer in Africa.



**Figure 1.4:** A map showing areas suitable for growing soybean in Africa (Source: IITA, 2009b).

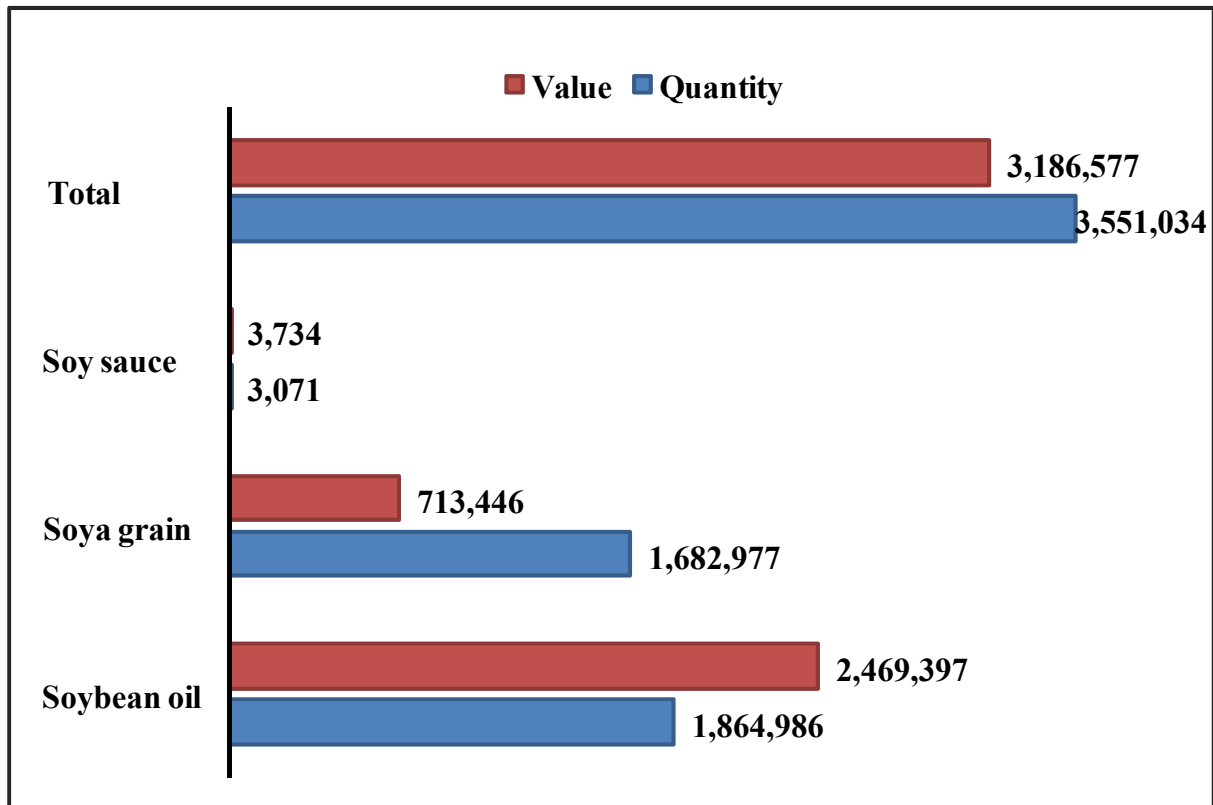
### 1.1.2 Consumption and economic importance

Grain legumes, such as peas, common bean, and soybean are rich in protein, starch, fiber and other essential nutrients for human nutrition and animal feed (FAO, 2003). Among these legumes, common bean plays substantial role in direct consumption by small-holder farmers in sub-Saharan African and therefore has a significant role in improving human nutrition (CIAT, 2010). Especially in areas with a high population density such as Rwanda and Burundi, about 80% of the production is used for home consumption (Wortmann et al., 1998). In these areas per capita consumption of beans reaches up to 36 kg/year (FAO, 2001).

Soybean plays an important role in nutrition in Africa among subsistence farmers (IITA, 2009a). This is due to the increased demand of soy cooking oil, soy-fortified food and animal feed (especially for poultry). The production gap for soybean in Africa is met by imports. In 2008, Africa imported 3.6 million tons of soybean worth about  $3.2 \times 10^3$  millions USD (Figure 1.5) (IITA, 2009a).

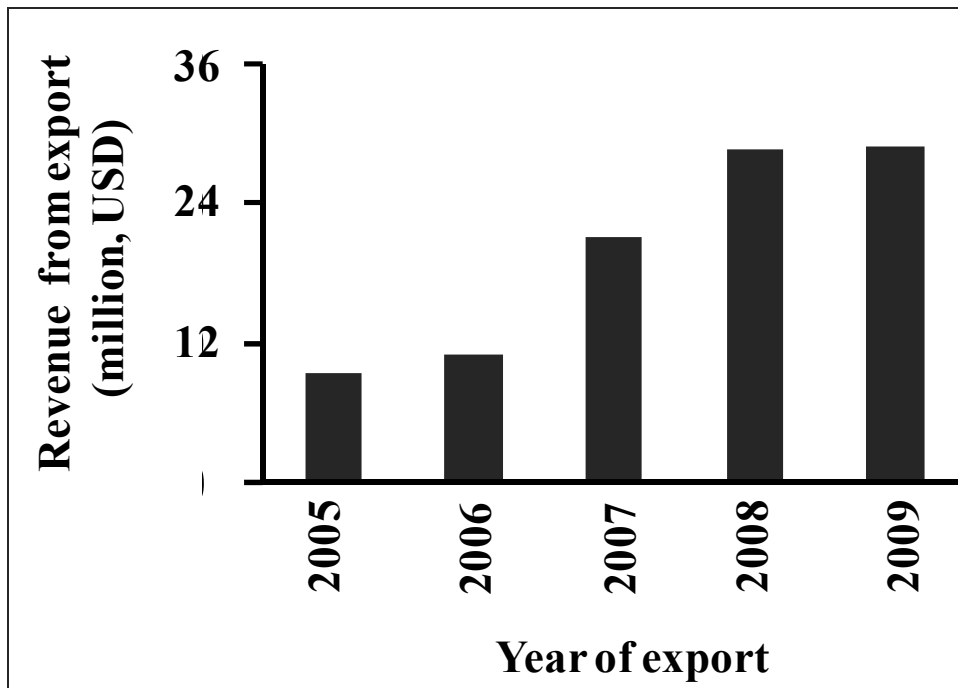
Common bean is a source of income for African economies by generating foreign exchange earnings and benefitting small-holder farmers. It has been estimated that Ethiopia's export earnings from common bean is greater than 20 million USD per annum (Beebe et al., 2010) with an increasing trend during the last five years (Figure 1.6, CSA, 2010). In contrast, soybean is not exported. Generally, the demand for both common bean and soybean will continue to increase due to a higher world-wide demand for ready-made or processed canned foods.





**Quantity (tons ) or value (1000USD)**

**Figure 1.5:** Import of soybean oil, grain and sauce into Africa for the year 2008 (Source: FAO, 2008).



**Figure 1.6:** Ethiopia's common bean export revenue for five consecutive years from 2005-2009

(Source: CSA, 2010).

### 1.1.3 Legumes and soil fertility

Legumes are also used as a natural nitrogen source in agriculture, particularly in Africa, through the presence of nitrogen-fixing bacteria in specialized organs (nodules) on the legume roots. Of the world's nitrogen demand, 60% ( $3 \times 10^9$  t N<sub>2</sub>) is met by symbiotic nitrogen fixation (SNF), followed by chemical fertilizer (25%) (Zahran, 1999b). This shows the importance of grain legumes in farming systems world-wide.

The contribution of nitrogen fixation for the two legumes (soybean and common bean), which are the focus of this study, is reported as [49-450 kg/ha N (Herridge et al., 2008; Wani et al., 1995)] and [57 kg/ha N (Wani et al., 1995) to 100 kg/ha N (Hardarson et al., 1993)], respectively. Therefore, the use of these legumes provides cheap natural fertilizer, which is also favoured in the increasing organic market, where legumes play a great role in providing much of the needed nitrogen for other subsequent crops. Soil nutrient depletion is a prominent problem for subsistence farmers. According to Graham and Vance (2003), the average depletion for 37 countries was 22 kg N/ha per year. Moreover, this problem is further aggravated by the low fertilizer use in Africa (FAO, 2003). Therefore, production of grain legume has a vital role in the cropping system by providing the cheapest, and one of the most effective ways, to maintain sustainable yields in African agriculture.

## 1.2 Legumes and drought stress

### 1.2.1 Importance of drought stress

Drought stress is the primary challenge for crop production globally. Grain legume production is severely constrained by drought (Grzesiak et al., 1996; Sincik et al., 2008; Sinclair et al., 2007). Drought poses considerable reduction of plant productivity and significantly threatens food security (Boyer, 1982) especially in areas where the agricultural system is dependent on rainfall. About one-third of the world's agricultural land currently suffers from chronically inadequate water availability (Boyer, 1982; Flexas et al., 2006a; Ghannoum, 2009). This situation is predicted to become progressively worse and is a formidable challenge for future crop production (Chaves and Oliveira, 2004; Jury et al., 2007).

Moisture stress accounts for high crop loss in common bean and soybean production in Africa. For instance, in more than 60% of bean growing areas in the world drought restricts common bean production (White and Singh, 1991b; Wortmann et al., 1998). Currently this estimation might increase due to climate change (Chaves et al., 2003). Annual loss of common bean due to drought stress in moisture stress areas of Africa has been estimated to be about 781,000 t (Wortmann et al., 1998). In common bean, drought causes a loss (from 4 to 10 t/ha grain yield) for areas with rain fall between 300-450 mm. Complete crop loss occurs in areas with rainfall of less than 300 mm (Wortmann et al., 1998). Figure 1.7 shows the importance of moisture stress in bean growing areas in sub-Saharan Africa. Due to the expansion of the production area, and also the influence of climatic change, the extent of areas affected by moisture stress is expected to

increase further. It has been estimated that 40% of current soybean producing areas, or those that are suitable for production in sub-Saharan Africa, mainly in Eastern and Southern Africa, is affected by water shortage (IITA, 2000). Research on drought tolerance is a major thematic area due to increased water stress that agricultural land may suffer in the future.

### 1.2.2 Research on drought stress

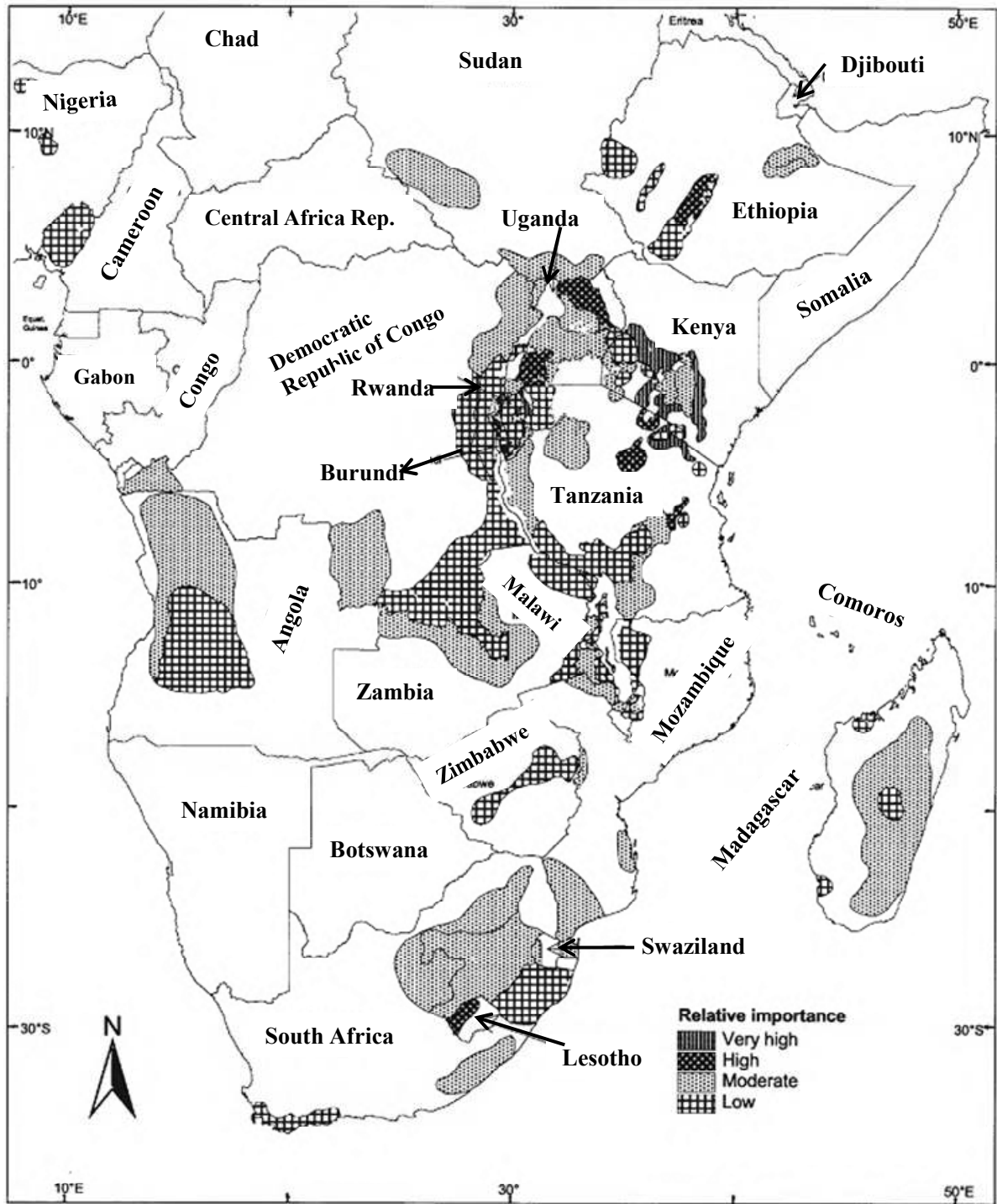
There are three common definition of drought (Whitmore, 2000; Wilhite and Glantz, 1985). Meteorological drought is defined as the prolong period when precipitation is significantly lower than the average value, which creates natural shortage of available water. Hydrological drought occurs when water reserves in the surface and sub-surface level (aquifers, lakes and reservoirs) decrease below the average value either due to shortage of rain or over consumption of water by humans. Agricultural drought occurs when the water supply (the precipitation and soil reserve) is unable to support crop production and restricts the expression of full genetic potential of the plant and causes reduction in crop yield (Turner and Brady, 1986). Based on time of drought prevalence and crop growth stage, agricultural drought can be further divided in to early season, mid-season, intermittent and late season (terminal) drought. However, terminal and intermittent drought are the most common type of drought affecting grain legumes production (Subbarao et al., 1995). These all suggests, the definition of drought is relative and differs based on the criteria and the concept of the water user (Whitmore, 2000).

Different types of drought adaptation mechanisms have been proposed. These are drought escape, drought avoidance and drought tolerance. Drought escape is defined as the ability of the

plant to complete its life span (flower, set pod, fill grain and mature) before the onset of water shortage (Beebe et al., 2010; Turner et al., 2001). In areas experiencing a short duration of rain fall or predicted to have terminal drought, varieties that escape drought perform better (Siddique et al., 1999; Thomson et al., 1997). Selection of early maturing grain legumes can have, however, undesirable impact on grain yield. Therefore, a variety with a flexible maturity might be more beneficial (Turner et al., 2001).

Drought avoidance is defined as the ability of the plant to sustain high tissue water content and potential under water limiting conditions. Drought avoidance strategies include reducing water loss by minimizing the expansion of leaf area and reducing stomatal conductance or by leaf movement / rolling. Further, better water absorption by extending root depth or increased root density and hydraulic conductance are also important mechanisms for drought avoidance (Manavalan et al., 2009; Morgan, 1992). Drought tolerance is defined as capacity of the plant to resist drought, i.e. decreased cellular water content or water potential. This may be achieved through osmotic adjustment which decreases cellular concentrations of osmolytes and increases water movement into the cell and tissue. This maintains turgor. Adjustment can be achieved through synthesis of compounds such as amino acids (proline) and sugars or other compatible solutes in the tissue or transported into it (Amede and Schubert, 2003; Jongdee et al., 2002; Nguyen et al., 1997). Such adaptations delay metabolic damage and leaf senescence and improves the transport of assimilate to the grain (Leport et al., 1999), thereby improving root development and water absorption (Morgan and Condon, 1986) and stomatal conductance and CO<sub>2</sub> assimilation. However, greater stomatal conductance will lead to faster water loss and so off-set the advantages of the adaptations.

Even though many researchers have been involved in drought related studies, the success is still very limited. Research has focused on the identification of common bean cultivars adapting to drought stress and promising drought-tolerant common bean lines as parental materials to create recombinant inbred lines parents (BAT 477, G21212, ICA Quimbaya and SEA 5) (Beebe et al., 2010). Although drought is one of the main production constraints for soybean in Africa, progress in developing drought-tolerant varieties is still slow with only a few promising soybean lines identified (Tefera, 2011).



**Figure 1.7:** Relative importance of soil moisture deficits in bean production areas of sub-Saharan Africa (Source: Wortmann et al., 1998).



Although past studies contributed to an understanding of drought stress tolerance, most of the studies were focused only on a specific plant performance trait. Moreover, the contribution of roots and the effect of water stress on symbiotic nitrogen fixation (SNF) have been less studied. O'Toole and De Datta (1986) stated that "drought is a syndrome" due to its complex behaviour and its uncertainty of prevalence, period and intensity of its persistence. Thus, success in developing a better performing legume under drought necessitates a comprehensive understanding of the physiological and morphological characteristics of above ground (shoot) and below ground (root) plant parts for drought tolerance as well as the symbiotic nitrogen fixing ability for the identification of main adaptation traits. This might help to develop selection criteria to support varietal improvement for devising effective breeding strategies for drought tolerance.

### 1.2.3 Physiological effect of drought on legume performance

White and Singh (1991b) defined drought in bean as, "the insufficiency of water availability during the growth cycle of the crop which limits the maximum expression of genetic potential". Therefore, developing better performing legumes under drought, exploiting the genetic potential of the existing germplasm and also breeding for enhanced tolerance, remains an important task for plant scientists.

### 1.2.3.1 *Leaf water potential*

Water deprivation leads to loss of water from the plant and changes the plant water status with a decline in the stomatal conductance and transpiration (Ribas-Carbo et al., 2005). Since leaf water potential is a good indicator of the plant water status (Turner, 1982), it is an important and dependable performance indicator in drought studies, and maintenance of the leaf water status is a key indicator for dehydration avoidance (Jongdee et al., 2002; Siddique et al., 2000). Reducing water loss through stomatal closure, rolling or abscission of the leaf, and increased plant water up-take through enhanced root development are mechanisms playing a role in maintaining the leaf water status (Jongdee et al., 2002). However, maintenance of leaf turgor through accumulation of solute have also been identified to be an adaptation strategy for maintaining the water status in legumes (Amede and Schubert, 2003). Plants which maintain the water status (high water potential) have been found to be productive under stress condition with low reproductive abortion (Jongdee et al., 2002; Pantuwan et al., 2002). Therefore, as leaf water status determines the gas exchange of the plant, it should be essential to use leaf water status as a selection indicator for drought.

### 1.2.3.2 *Photosynthesis*

The gas exchange of the plants (as reflected in CO<sub>2</sub> assimilation, transpiration and stomatal conductance) is the principal plant process responsible for plant biomass production and for plant adaptation to a change in environment (Lawlor and Tezara, 2009a). Stomatal opening, which controls gas exchange, is a sensitive indicator for drought stress. In common bean, and most other legumes, stomatal closure for reducing the water loss is one of the adaptation strategies to drought (Miyashita et al., 2005). However, stomatal closure also results in reduced CO<sub>2</sub> movement for carboxylation within the chloroplast. This can be a major cause of drought-induced decreases in CO<sub>2</sub> assimilation capacity, particularly in C<sub>3</sub> plants (Chaves and Oliveira, 2004; Flexas et al., 2006b; Warren, 2008), and causes reduced leaf expansion and plant biomass production (Chaves et al., 2003; Lawlor and Tezara, 2009a). Furthermore, during the process of photosynthesis, photochemical and biochemical activities occur in the leaf and these activities are seriously affected by drought stress (Chaves et al., 2002). Since CO<sub>2</sub> assimilation is the major factor in the plant's supply of carbon and ATP, and is susceptible to drought (Lawlor, 2002a; Parry et al., 2002), measurement of CO<sub>2</sub> assimilation will continue to be a major target in drought stress studies. Further, since the adaptive strategies of legumes for water deficit are either stomatal closure or heliotropic leaf movement (Pastenes et al., 2005), legumes which can sustain stomatal conductance or have enhanced CO<sub>2</sub> assimilation per unit of stomatal opening will be a target for future plant improvement programs. Also, complementing existing information on gas exchange performance and understanding the relationship with other performance traits will be vital for using these traits for evaluation of legumes in future varietal improvement program. Existence of genetic variability for gas exchange performance in

common bean (Comstock and Ehleringer, 1993; Gebeyehu, 2006; Mencuccini et al., 2000) and soybean (Fenta et al., 2011; Flexas et al., 2006a; Liu et al., 2005) has already been reported

### 1.2.3.3 Water use efficiency

Water use efficiency has several definitions and varies depending on scale, e.g., plant leaf or whole plant or time, e.g., short-time scale of minutes or longer-term up whole plant growth season (Bacon, 2004). For the whole plant over the growth season, water use efficiency is defined as the ratio between production of biomass, shoot biomass or harvested yield and total evapo-transpiration or plant transpiration (Chaves and Oliveira, 2004; Connor et al., 1992). For the plant leaf, WUE is defined as the ratio between instantaneous net CO<sub>2</sub> assimilation rate (A) and transpiration (E) (A/E). Since A/E largely depends on vapour pressure deficit, the ration between CO<sub>2</sub> assimilation and stomatal conductance (G) (A/G), which is termed intrinsic water use efficiency, is usually used as a normalized value when compared to instantaneous water use efficiency (Chaves and Oliveira, 2004; Farquhar et al., 1989; Soares-Cordeiro et al., 2009b). Intrinsic water use efficiency provides a direct measure of activity of the photosynthetic system normalized to constant stomatal conductance. The use of CID (carbon isotope discrimination) also provides a measure of intrinsic water use efficiency and primarily measures the ratio of A/G (Bacon, 2004).

Using CID is simple and rapidly estimates WUE over time (Farquhar et al., 1982). This concept was further supported by CID research work in higher plants by Evans et al. (1986), who established as CID reveals CO<sub>2</sub> assimilation of the weighted average throughout the growth

period of the plant. Furthermore, in the field experiments application of WUE needs measurement of whole plant or field level crop water use, apart from its tediousness, it is labour and time consuming. The innovation of CID as a heritable trait for understanding the gas exchange mechanism in plants at the whole plant level as an estimate of WUE simplified the measurement of WUE at the field level (Farquhar et al., 1989; Hall et al., 1998). Additionally, the correlation of lower CID value measured at the leaf as well as grain with higher WUE in most studies (Blum, 2011) further suggest the importance of the use of CID for WUE measurements. For this reason, CID measurement has become one of the selection traits for drought screening in maize and wheat at CIMMYT (Pask et al., 2012). So far, CID has not been widely applied in a legume improvement program.

Traits that serve to conserve water (conservative traits) include low stomatal conductance, low leaf growth rate, or deep root systems provides better water use efficiency. Research often focused on the use of instantaneous water use efficiency (IWUE) values as a physiological marker for drought tolerance. Higher IWUE values indicate improved tolerance to drought since varieties with high IWUE values are better able to assimilate carbon at low stomatal conductance and hence attain a greater yield using less water. Confirmation of a direct relationship of WUE with  $A/G$ ,  $C_i/C_a$  (ratio of intracellular to ambient air  $CO_2$  concentration) and with carbon isotope discrimination ( $\delta^{13}C$  measurement) provides a tool for WUE evaluation (Farquhar and Richards, 1984; Farquhar et al., 1982; O'Leary, 1988). The biochemical basis for  $\delta^{13}C$  measurement in  $C_3$  plant is due to the inherent discrimination of  $^{13}C$  by ribulose-1-5-biphosphate carboxylase (RuBPC-ase) in favour of  $^{12}CO_2$  (by a factor of  $\sim 27\%$ ), because of lower reactivity of  $^{13}C$  (Farquhar et al., 1982; Farquhar et al., 1989). CID ( $^{13}C/^{12}C$ ) has been applied for WUE

efficiency evaluation in several crops (Farquhar and Richards, 1984; Farquhar et al., 1982; Martin and Thorstenson, 1988; Rytter, 2005; Saranga et al., 1998). This allowed evaluation of a large number of germplasm for WUE under controlled and field experiments measuring the amount of water consumed by the plant (Rytter, 2005).

Due to the existence of a negative association of CID with WUE in several different  $C_3$  plants (Farquhar et al., 1989) including wheat (Condon et al., 1990; Farquhar and Richards, 1984), alfalfa (Johnson and Rumbaugh, 1995; Johnson and Tieszen, 1994), cool season grass (Johnson and Asay, 1993) and barley (Anyia et al., 2005), low CID has been used as a selection criterion for enhanced WUE (Shaheen and Hood-Nowotny, 2005). Moreover, the negative association of CID with plant biomass and seed yield for drought tolerant cereals (Anyia et al., 2005; Condon et al., 2002; Shaheen and Hood-Nowotny, 2005; Teulat et al., 2001) supports the importance CID as a selection criterion for enhanced WUE.

Variation for WUE has been observed in soybean (Hufstetler et al., 2007) and in common bean (Ehleringer, 1990; Gebeyehu, 2006) regarding the amount of dry matter produced per given amount of water and CID. More importantly, the moderate to high heritability of CID found in cowpea (Menéndez and Hall, 1996), common bean (Ehleringer, 1988), wheat (Araus et al., 1998) and cool season grasses (Johnson et al., 1990) provides additional support of the use of CID for water stress evaluation. However, breeding plants for high WUE under drought might actually result in low-yielding plants (Blum, 2011) when WUE is not associated with productivity traits (Menéndez and Hall, 1996). Therefore, the usefulness of IWUE or CID as a

selection parameter depends on its contribution to productivity and association to productive traits under drought (Menéndez and Hall, 1996).

#### 1.2.3.4 Plant growth, biomass and productivity

Of the wide range of possible morphological characteristics that can be used in the selection of legume varieties for enhanced drought tolerance, shoot parameters are generally considered to be the easiest to assess under field conditions. Shoot markers remain major targets in breeding programs, particularly in developing countries, where variations in shoot morphology are often determined subjectively under field or glasshouse conditions (Manavalan et al., 2009). Often this involves monitoring leaf area (Mohamed et al., 2002), dry matter yield per plant (Mohamed et al., 2002; Udensi et al., 2010), harvest index, and finally grain yield (Gebeyehu, 2006; Muñoz-Perea et al., 2007).

Leaf shading and reduced leaf elongation (Acosta Gallegos, 1988) through inhibition of cell division and expansion (Zhu, 2001), adjustment in days to maturity and rapid biomass accumulation (Siddique et al., 2001) have been found to be adaptive strategies under drought stress. Reducing biomass or leaf area to minimize stomatal opening and reducing transpiration rate is important for terminal drought but these characteristics might be less favourable for a longer drought period resulting in less plant biomass and seed yield (Blum, 2011). Slow plant growth for better assimilate partitioning and production of protective compounds (Zhu, 2002) as well as enhanced root development for maintaining plant water status and cellular activity (Chaves et al., 2003) have also been found to be adaptation traits for drought stress that

contribute to more harvestable yield. Therefore, drought avoidance through maintaining the plant function might be a target for selection for breeders to improve plant productivity under stress condition (Chaves et al., 2003).

Improving plant productivity under drought condition requires selection for a higher biomass accumulating genotype (Lopes et al., 2011), since biomass and grain yield have a strong positive association, especially in grain legumes (Ramirez-Vallejo and Kelly, 1998; Shenkut and Brick, 2003). Furthermore, previous studies also ascertained the high heritability of plant biomass (Shenkut and Brick, 2003). As in other crops, legume biomass and productivity has been found severely restricted by drought stress and these traits can be used as selection markers for performance under drought (Beebe et al., 2010). Plant productivity is also a function of the amount of interception of photosynthetically active radiation (PAR). Zhu et al.(2010) estimated the efficiency of PAR interception and the effectiveness of converting assimilate into biomass and grain yield. Plant performance is further associated with effective use of water through better root development as a drought avoidance mechanism (Blum, 2011). Since higher root development can be a trade-off for shoot as well as grain production, the cultivar which maintains a better root: shoot ratio has an advantage. However, this is highly related to the nitrogen use as well as photosynthetic efficiency of the plant (ÅGren and Franklin, 2003; ÅGren and Ingestad, 1987). In addition, understanding the relationship of these productivity traits with other performance traits will also be vital to determine the contribution of different parameters to yield.



### **1.3 Effect of drought on roots**

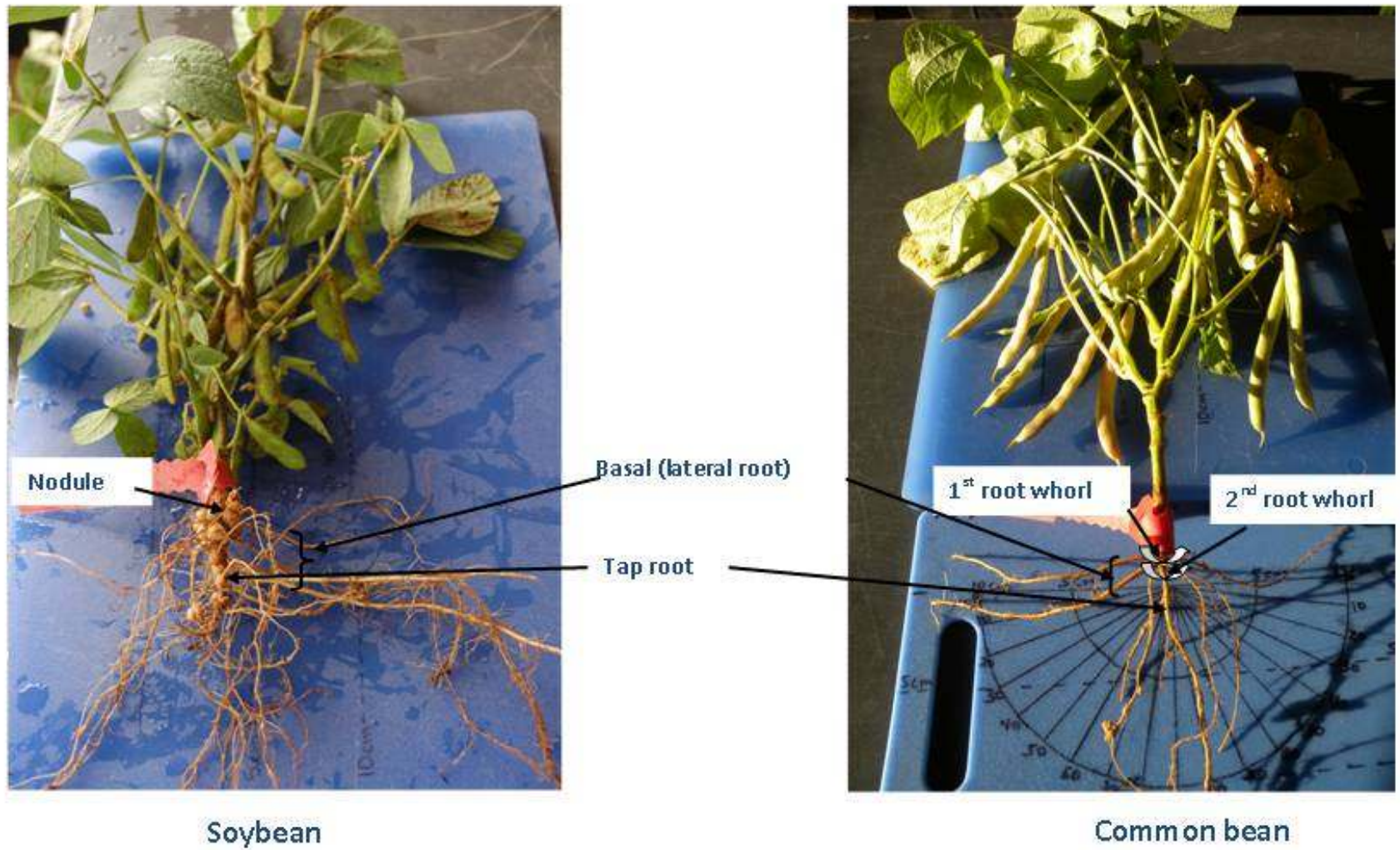
#### **1.3.1 Root system of legumes**

The root system is the principal plant organ which provides absorption of water and essential nutrients from the soil (Malamy, 2005). However, since these soil resources are limited and differ depending up on the soil type and environmental factors, the survival of the plant depends mainly on the inherent root morphology and architecture along with the modulation of the root structure in response to the external stimuli (Fitter, 1987). As common bean and soybean are mainly grown in water-limited tropical and sub-tropical areas in Africa, root traits play a fundamental role for adaptation as well as for enhanced productivity (CIAT, 2007; IITA, 2009a).

In general, root morphology refers to the external features of root. These include characteristics such as root length, diameter, area, volume and number of root tips (Lynch, 1995; Regent Instruments Inc., 2011). Root architecture is the spatial configuration (three dimensional structures) of the root system at a point in time, considering the different parts of the root system (tap root, lateral roots and root hairs) (Lynch, 1995; Lynch, 2007; Osmont et al., 2007). The root system in flowering plants is classified into two types. The allorhizic root system is the first type and is commonly found in the dicot plants (Osmont et al., 2007). Soybean and common bean also have this typical root system. In the allorhizic root system there are two main types of roots, the primary root (tap root) and lateral (basal) roots (Figure 1.8). The first root that emerges from the embryo is the tap root. The other roots, which emerge from the tap root and which can produce the higher order roots (branches), are the lateral (basal) roots (Esau, 1965; Larcher, 2003). The basal roots in common bean emerge in a circular pattern at the point of initiation of

the tap root and this pattern is called root whorl. The number of whorls might vary across different genotypes mostly from 1 to 3. The lateral roots are considered to be basal when it originated from primary root (radicle) at early germination stage. The sequence of the whorls is from the top to the base of the tap root (Figure 1.8). The allorhizic root system has also adventitious roots. These roots emerge above the root whorls (basal roots) from the stem or hypocotyls. The second type of root system is the homorhizic root system which is common to monocot plants (Esau, 1965; Larcher, 2003).

The configuration of the root organ is quite stable and controlled by inherent genetic factors of plant species. However, the amount, placement and direction of root growth vary, even within the same species (Malamy, 2005). The extent of developmental plasticity of the root system for these diverse characteristics of the root in the soil environment is controlled by hormones responding to external stimuli (Bao et al., 2004). In particular, auxin has a major role in controlling the root development in plants (Lucas et al., 2008). The emergence as well as the growth and development of lateral roots are both controlled by auxin (Casimiro et al., 2001; De Smet et al., 2007). In legumes, abscisic acid (ABA) has a role for lateral root development (Liang et al., 2007). Under drought condition, both ABA and water stress synergistically enhance the lateral root development and contribute to drought performance (Xiong et al., 2006). However, enhanced ABA activity during drought stress has a negative impact on the legumes nodule number.



**Figure 1.8:** Soybean and common bean plants with major root types.

### 1.3.2 Root architecture and morphology

Advances towards improvement for drought tolerance have mainly been based on the evaluation of above ground (shoot) traits. The complexity of drought stress and the rather little activity in root research has limited major advances in drought stress tolerance in legumes (Blum, 2005). This might be due to the difficulty in measuring the below ground root system architecture, such that most plant scientist are reluctant to work with roots (Nielsen et al., 1997).

Drought affects the development of root architecture due to its role as an initial sensor organ to water deficit. Plants generally modify their root architecture and increase total root absorption surface area by new lateral root formation and elongation as a mechanism for avoiding drought stress (Osmont et al., 2007). Unlike other dehydration avoidance strategies (stomatal closure, leaf rolling or abscission), dehydration avoidance through improved root development sustains productivity of plants by maintaining the plant water status and photosynthetic assimilation (Lopes et al., 2011). Previous research has also shown the importance of deep rooting for better performance under drought in beans (White and Castillo, 1991), wheat (Reynolds et al., 2007) and rice (Li et al., 2005). Therefore, better understanding of root traits would be vital for improving the legume selection strategy to sustain productivity under water-limited conditions. Other research groups have also demonstrated the importance of nitrate for root development and nodulation (Gresshoff, 2010). Therefore, this suggests the requirement of not only searching for better root development under drought, but also performance under nitrate starvation or SNF ability.

Research has further shown the existence of genetic variability in common bean for phosphorous stress and in particular for basal and tap root development (Beebe et al., 2006) as well as for root density and branching (Lynch, 2007). Common bean also expresses genetic variability in root growth in response to water deficit with deep rooting ability or enhanced root mass (Sponchiado et al., 1989; White and Castillo, 1991). In soybean, root architectural (root diameter and length) (Ao et al., 2010; Zhao et al., 2004) and morphological traits (root volume, area and length) (Ao et al., 2010) have also been investigated as an adaptive strategy for drought stress.

Despite the existence of variability in legumes for root traits, the use of these traits in a plant improvement program still needs to be confirmed as a valuable contribution to productivity. The contribution of root traits to plant growth and productivity has been investigated in common bean (Sponchiado et al., 1989) and chickpea (Kashiwagi et al., 2006). There is a relation between root features and plant productivity in terms of yield, water use or nutrient capture (Steele et al., 2007). Evidence of a relationship between root architecture and plant productivity, water use or nitrogen use efficiency (Garnett et al., 2009) would also provide insights into the relationship of root system architecture with symbiotic nitrogen fixation (SNF) traits. This was therefore a major focus of this PhD study.

The production of plant biomass and grain yield under drought also depends on the maximum moisture capture to satisfy the transpiration demand. This efficiency is termed “effective use of water (EUW)” (Blum, 2011). Oxygen isotope enrichment ( $\delta O_2$ ) has been found to be a good proxy for the transpiration rate providing information about the amount of water supplied by root development to meet the evaporative demand (Sheshshayee et al., 2005). Moreover, the existence

of a positive association of root mass with  $\delta O_2$  (Elazab et al., 2012) further indicates that root traits are useful indicators for EUW. Also, the adverse effect of drought in plants is effectively avoided by changing assimilate allocation to allow for better root development before the onset of the adverse effect on plant development (Lopes et al., 2011). Further, root development and nodulation are highly dependent on the soil moisture availability (Garside et al., 1992).

### 1.3.3 Effect of drought on nodules

Legumes are highly important in supplying nitrogen through symbiotic nitrogen fixation (SNF) (Herridge et al., 2008). Legumes nodules are formed due to the symbiotic interaction of the legume and bacteria (Serraj et al., 1999) in common bean (*Rhizobium leguminosarum* bv. *phaseoli*) and soybean (*Bradyrhizobium japonicum*). Both these tropical legumes produce a determinate type of nodule. Photosynthetic assimilates and other metabolites are transported to the nodule through diffusion from the phloem in the nodule cortex. The SNF products, usually ureides (allantoin and allantonic acid), are transported to the shoot via the xylem (Schubert et al., 1995). However, nodule infection to establish a symbiosis as well as the bacterial activity are severely restricted by drought (Kirda et al., 1989). Generally, drought reduces the quantity of rhizobial bacteria in the soil, and their development and infection ability. This affects the formation of nodules and synthesis of leghemoglobin. Finally, the nodule life span and SNF are severely affected by drought (Hungria and Vargas, 2000; Venkateswarlu et al., 1990).

Reducing  $N_2$  by the nitrogenase in the SNF process consumes high energy derived from photosynthesis as shown in the equation below (Serraj et al., 1999):



Further, according to (Rainbird et al., 1984) to sustain nitrogenase for nodule growth and for N transport, a total of 12.2 g of carbohydrate per g of N fixed is used by soybean nodules. SNF is therefore an energy demanding process. Any process which limits plant photosynthesis for supplying carbohydrate also affects SNF. Under water deficit, reduced carbon assimilates further affects SNF efficiency in legumes. Also, the high energy demand of the nodule requires a high nodule flux of oxygen for respiration. Therefore, maintaining and regulating the demand and supply of oxygen by the nodules during drought is a challenge. Nitrogenase is inhibited by O<sub>2</sub> (Minchin, 1997). To ensure proper function of root nodules, leghemoglobin in root nodules carries oxygen and also buffers free oxygen in the cytoplasm of the nodule cells. Since leghemoglobin stores oxygen for optimal nodule respiration and transport to respiring symbiotic bacteria for a few seconds (Denison and Harter, 1995), a continuous supply of oxygen for functioning is required. A decline in oxygen permeability due to the creation of oxygen diffusion barrier results in reduced SNF activity (Denison, 1998). Therefore, any decline in the oxygen flux caused by the drought as a plant response reduces SNF activity of the legumes (Arrese-Igor et al., 2011).

#### 1.3.4 SNF and plant biomass and productivity

SNF, measured as the amount of N accumulation, is sensitive to drought (Serraj et al., 1997; Sinclair, 1986). In field experiments, drought reduced SNF (N accumulation in the shoot) in soybean by 56% and biomass by 42% relative to well-watered controls (King and Purcell, 2006). Drought affects biomass and SNF ability in most legumes including common bean (Castellanos

et al., 1996b) and mung bean (Thomas et al., 2004) with SNF more affected than biomass. However, decrease in N-accumulation is not only due to the decline in SNF ability, but is also a consequence of a decreased biomass amount (Streeter, 2003). This suggests a significant role of SNF in maintaining plant biomass and *vice versa*. Further, a strong association between seed yield and different SNF performance traits, such as  $^{15}\text{N}$  natural abundance, nodules mass and number has been found (Pazdernik et al., 1997; Ronis et al., 1985). Also, genetic variability associated with various degrees of sensitivity of SNF to drought has been found for soybean (King and Purcell, 2006; Pazdernik et al., 1996) and common bean (Castellanos et al., 1996b; Sinclair and Serraj, 1995).

### 1.3.5 Methods of SNF measurement

#### 1.3.5.1 $^{15}\text{N}$ analysis

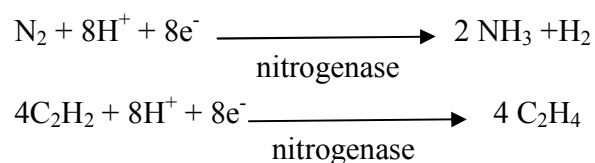
Several methods have been used for quantifying  $\text{N}_2$  fixation ability of legumes. This includes  $^{15}\text{N}$  natural abundance and nitrogenase activity, or acetylene reduction assay (ARA) techniques. In nature, there are two principal isotopes of nitrogen:  $^{14}\text{N}$  and  $^{15}\text{N}$ . Even though the chemical characteristics of these N isotopes are similar, they have small quantitative differences due to their difference in mass and activation energy. In nature, the lighter isotope  $^{14}\text{N}$  is highly abundant. The isotope  $^{15}\text{N}$  represents 0.36663% of atmospheric nitrogen and this concentration is termed “natural abundance” (Högberg, 1997). Therefore, if the  $^{15}\text{N}$  concentration in the atmosphere differs from the plant available soil N, and these values are known,  $\text{N}_2$  fixation can be calculated on the basis of  $^{15}\text{N}$  analysis of a N-fixing legume and a non-fixing plant. This



difference is expressed as  $\delta^{15}\text{N}$  parts in thousands (‰) relative to  $^{15}\text{N}$  of the atmosphere (Shearer and Kohl, 1986). This method is used for SNF performance measurement in common bean (Castellanos et al., 1996b), soybean (King and Purcell, 2006) and also mung bean (Thomas et al., 2004). Although this SNF measurement method needs specialized equipment (mass spectrometer) and the cost associated with this measurement can be expensive, the technique is considered to be a direct measurement of  $\text{N}_2$  fixation (Peoples et al., 1989).

#### 1.3.5.2 Nitrogenase activity assay (ARA)

The principle behind the acetylene reduction assay is that nitrogenase, found in the  $\text{N}_2$ -fixing bacteria, reduces  $\text{N}_2$  to ammonia ( $\text{NH}_3$ ) in legume nodules. This enzyme is also able to reduce acetylene ( $\text{C}_2\text{H}_2$ ) to ethylene ( $\text{C}_2\text{H}_4$ ). Therefore,  $\text{C}_2\text{H}_4$  can be used as an alternative substrate of nitrogenase (Hardy et al., 1973). By placing nodule roots, either detached or with the plant root system, in an air tight vessel and incubating with  $\text{C}_2\text{H}_2$ , the amount of  $\text{C}_2\text{H}_4$  produced over a certain period is quantified using a gas chromatograph. This can be expressed in the following equations:



This method is considered to be an indirect method of assessing SNF by determining enzyme activity based on the electron flux through nitrogenase. The method is a simple, rapid and very sensitive analytical method for detecting nitrogenase activity. However, variation of enzyme

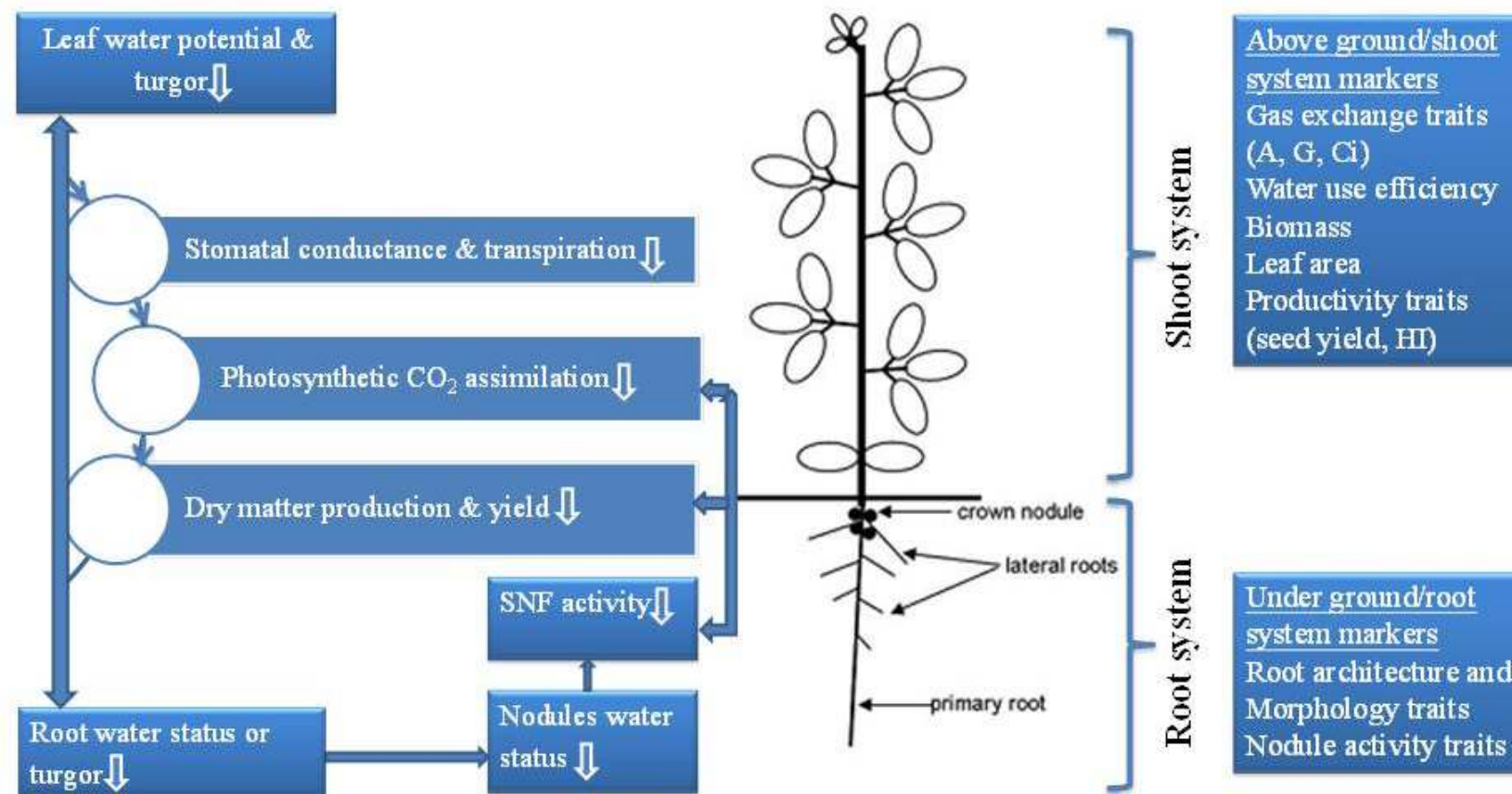
activity in intact and detached material necessitates taking several measurements (Peoples and Herridge, 1990). This method has been used for SNF measurement in faba bean (Plies et al., 1995) and also ten other legumes including common bean, soybean (Sinclair and Serraj, 1995) and chickpea (Thavarajah and Ball, 2006).

Apart from evaluation of legumes using natural abundance of  $^{15}\text{N}$  and ARA, several other nodule performance parameters such as nodule number, nodule mass (Fenta et al., 2011; Pazdernik et al., 1996) or nodule scoring have been used. These parameters have a direct relation with SNF. Complimenting data with  $^{15}\text{N}$  natural abundance might provide a better understanding of the performance of legumes. Using these methods in combination with other nodule performance parameters as well as morpho-physiological performance traits could contribute to a more thorough evaluation of the performance of legumes under water deficit.

#### **1.4 Rationale for study**

Drought has a significant influence on overall performance of grain legumes as summarized in Figure 1.9, and cause significant yield loss. There is however a possibility to minimize the problem through appropriate research. Identification and measurement of plant performance traits related to drought are fundamental to select superior performing legumes in drought affected areas. Although extensive research has been conducted for searching aboveground physiological and morphological traits for better performance under drought, plant root architectural plasticity and the role of symbiotic nitrogen fixation have not been investigated so far in great detail. These suggests, sustainable grain legume production can be achieved through investigation and

application of physiological adaptation mechanism through systematic (root and shoot plant performance) approach (Figure 1.9) and integrating these finding to the existing legume improvement program. Therefore, for understanding both underground (root system) and the aboveground (shoot) basis of plant performance, identification of critical plant morpho-physiological performance traits (markers) for drought tolerance is still urgently needed. This study sought to evaluate the potential of such markers to select the best performing lines under drought, by comparing different common bean and soybean lines.



**Figure 1.9:** Summary schematic diagram of the possible effect of water stress on legumes overall performance and suggested phenotypic markers for legumes performance evaluation. Downward arrows indicate decrease compared to the well-watered state  
 SNF: symbiotic nitrogen fixation, A= CO<sub>2</sub> assimilation, G=Stomatal conductance, Ci= Intra-cellular CO<sub>2</sub> concentration, HI=harvest index.

## 1.5 Working hypothesis and aim of the study

The overall scientific hypotheses of this study were that:

- 1) Both common bean and soybean have a similar morpho-physiological phenotypic basis of drought adaptation allowing the application of identical performance markers for selection of drought tolerant cultivars under both controlled and field growth conditions.
- 2) Root and nodule markers associated with SNF would allow the the best performing common bean and soybean cultivar under drought to be selected.
- 3) There is a direct relationship between water use efficiency, measured by carbon isotope discrimination, and symbiotic nitrogen fixation, determined by  $^{15}\text{N}$  natural abundance,
- 4) Water use efficiency and symbiotic nitrogen fixation are strongly correlated with seed yield under well-watered and drought conditions .
- 5) Plant performance under well-watered and drought condition is identical under controlled environmental conditions and field conditions.

The overall aim of this PhD study was to investigate the performance of different bean and soybean cultivars under drought, and to evaluate selected morphological or physiological phenotypic traits (markers) for their potential as markers to select superior cultivars under drought.

The specific objectives of the study were (i) to determine performance of different bean inbred lines and soybean cultivars under water deficit conditions to identify easily measurable plant performance parameters that are associated with drought tolerance under controlled growth

condition. (ii) to identify root and nodule markers associated with SNF that will allow the selection of the best performing cultivar under drought (iii) to verify if there is a direct relationship between the carbon isotope discrimination as well as  $^{15}\text{N}$  natural abundance with seed yield, root traits and nodule performance under well-watered and drought conditions in common bean lines (iv) to evaluate root and shoot traits under drought stress to determine the physiological basis of differences in growth and seed yield of three soybean cultivars and (v) to evaluate if data obtained by growth of common bean and soybean cultivars in a phytotron are comparable to field data.