

**Mungbean [*Vigna radiata* (L.) Wilczek]: Protein-rich legume for
improving soil fertility and diversifying cropping systems**

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Mungbean [*Vigna radiata* (L.) Wilczek]: Protein-rich legume for improving soil fertility and diversifying cropping systems

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ABSTRACT

Drought, salinity, and low soil fertility have negative impacts on agricultural productivity, resulting in food scarcity and nutritional insecurity, particularly in Sub-Saharan Africa. Mungbean [*Vigna radiata* (L.) R. Wilczek] has seen increased interest as a short-duration and drought tolerant legume crop, capable of atmospheric N₂ fixation. Mungbean is a protein and iron-rich legume and can be used as vegetable or grain for human consumption or multipurpose crop. At present, few studies have simultaneously explored the best agronomic practices for mungbean cultivation and evaluated its potential for increasing crop yields via intercropping systems and improving soil fertility through biological N₂ fixation. To understand the agronomic practices and soil physical properties limiting mungbean production, the impacts of two mungbean cultivars (Berken and OK2000) with and without inoculation with *Bradyrhizobium* spp. grown in loamy sand and silt loam soils on mungbean growth and yield were investigated under glasshouse conditions. Promising results from this study led to the introduction of mungbean into pearl millet systems in Senegal and evaluation of the effects of intercropping on growth, yields, land equivalent ratio (LER), canopy cover estimates, and normalized difference vegetation index (NDVI). Finally, we evaluated plant growth and N₂ fixation of five mungbean genotypes grown in two soil textures using the ¹⁵N natural abundance technique leading to recommendations for those with the greatest overall benefit to the cropping system.

The literature review shows mungbean often proposed as a strategic crop for increasing legume diversification within current cropping systems and providing increased

food security as well as market diversification and economic sustainability. The greenhouse study revealed that OK2000 cultivar produced significantly higher yield when inoculated and planted on a silt loam soil than other treatments, indicating the importance of inoculation and soil texture in mungbean establishment. Intercropping mungbean and millet significantly ($p \leq 0.05$) increased combined yields (35% to 100% increase) and LER compared to sole millet cropping systems. Canopy cover estimates and NDVI values significantly increased up to 60% and 30%, respectively, in millet-mungbean intercropping over millet alone. The N_2 fixation study showed that %Ndfa of mungbean was higher when grown in the loamy sand soil (27% increase). However, soil N uptake ($235 \text{ mg plant}^{-1}$) and amount of N fixed (67 mg plant^{-1}) were greater in the silt loam soil. Among genotypes, IC 8972-1 significantly ($p \leq 0.05$) derived less N from the atmosphere (23%) but took more soil N ($155 \text{ mg plant}^{-1}$) which yielded significantly greater dry biomass ($7.85 \text{ g plant}^{-1}$) and shoot N content ($200 \text{ mg plant}^{-1}$). The results from the N_2 fixation study indicated that choice of mungbean genotype can contribute to reducing N needs of agricultural systems. Overall, this research project demonstrated that mungbean has the potential for diversifying smallholder agriculture and adding biologically fixed N into soils, in line with transformative adaptation strategies being promoted for sustainable agriculture. Further research and development programs on good cultural practices, adaptation to cropping systems, and nutritional benefits for human consumption can promote mungbean cultivation in SSA.

Mungbean [*Vigna radiata* (L.) Wilczek]: Protein-rich legume for improving soil fertility and diversifying cropping systems

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GENERAL AUDIENCE ABSTRACT

Global population growth is expected to reach 9.8 billion in 2050 while climate change is predicted to reduce food production. Sustainable solutions are needed for increasing food availability and satisfying nutritional needs under changing climatic conditions. Mungbean is a viable option because it is a legume crop capable of restoring soil fertility and has low water requirements. Mungbean also contains high levels of protein and iron and can, therefore, provide a nutritious and healthy food. Although the agronomic benefits of mungbean have been studied, best cultural practices and its impact on farming systems and soil fertility are scattered. The objectives of this research were to identify the best agronomic practices for mungbean production, assess its effects when grown together with millet, and measure its nitrogen contribution to the soil. The results showed that selecting the best genotypes to be grown in a particular soil texture can significantly increase mungbean growth and yield. In addition, incorporation of mungbean into cereal-based farming systems demonstrated its capacity for improving agricultural production in a low-input environment. Assessment of nitrogen fixation by mungbean showed that it can naturally add nitrogen into the soils, the most limiting plant nutrient, reducing nitrogen application needs. Thus, the ability of mungbean to diversify farming systems, improve soil fertility, and deliver nutritious food will provide agronomic, environmental, and economic benefits to farmers, especially in food-insecure households. However, exploitation of the full potential of mungbean won't be achieved without understanding the major factors influencing mungbean cultivation and production.

DEDICATION

To my parents, sister, brothers, and wife,

you certainly deserve better but I tried to do my best,

which you always advised me to do;

To my brother Bonaventure Lambal *in memoriam*,

gone forever, obedience is the memory that I keep of you;

I dedicate this modest work.

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Chapter I – Introduction

Context and justification

Climate change has been projected to reduce agricultural productivity globally, but especially across sub-Saharan Africa (SSA) where severe droughts negatively impact the livelihoods of rural populations (Ramírez Villegas and Thornton, 2015; Tadele, 2017). The vulnerability of the SSA region is due to its dependence on rain-fed agriculture and natural resources, high vulnerability to climate variability (Roudier et al., 2011; Niang et al., 2014), low soil fertility (Connolly-Boutin and Smit, 2016; Vanlauwe et al., 2019), and low focus on transformative adaptation strategies to climate change (Grist, 2014).

Most of the research on increasing agricultural production has focused on improving yields of cereal crops, but not on developing transformative adaptation strategies for resilient multi-crop production systems that can improve the livelihoods of the most vulnerable populations. The literature about agriculture in SSA shows that food security is dependent not only on crop production but also on nutritional security. Research on sustainable intensification of production systems demonstrates that adaptation and mitigation strategies must address the challenges of agricultural productivity, climate change, food security, and poverty. The aim of this research project is to assess the potential of inclusion of mungbean, a legume crop, for diversifying cropping systems to promote sustainable adaptation strategies and reduce food insecurity.

Legumes for sustainable intensification

The important role of legumes for diversifying and intensifying production systems and ultimately promoting sustainable agriculture has been extensively reviewed by several authors (Franke et al., 2018; Snapp et al., 2019; Vanlauwe et al., 2019). Legumes, in symbiosis with rhizobia, can fix atmospheric nitrogen, thus contributing to improve soil fertility and reduce inorganic nitrogen fertilizer needs (Giller, 2001; Smith et al., 2016). Legumes can also reduce N₂O emissions compared to cereal crops and decrease the carbon footprint associated with the production of protein, making legume crops a mitigation strategy to greenhouse gas emissions from agriculture (Foyer et al., 2016; Magrini et al., 2016). Legumes in association or rotation with cereal crops have also resulted in improved nitrogen-use efficiency and soil phosphorus uptake as well as enhanced soil physical and biological properties, thus increasing combined seed yields (Li et al., 2009). Other reported benefits include reduction of insect pests, disease and weed incidence (Matusso et al., 2014). Legume crops are also a cheap and available source of protein and minerals which could help in reducing malnutrition in SSA (Nair et al., 2013; Dahiya et al., 2015; Pataczek et al., 2018).

Yet, despite these agronomic, environmental, and nutritional benefits, cultivation of legume crops still remains low in Sub-Saharan Africa, particularly in West Africa (Vanlauwe et al., 2019). Although, research has demonstrated the advantages of including legumes into cropping systems, little to no-research has focused on the potential of introducing new legume crops to new localities such as mungbean to Senegal. In this dissertation, I hypothesize that: 1) inoculation with *Bradyrhizobium* spp. improve plant growth, nodulation and yield components, and the magnitude of this effect will vary by cultivar and soil texture; 2) optimized density of mungbean in millet-based systems will increase the growth and yields and plant health status

compared to monoculture; and 3) selected high yielding and nutrient-dense cultivars will exhibit greater potential for N₂ fixation.

Mungbean is a short-duration and drought-tolerant legume crop capable of improving soil fertility through biological N₂ fixation, maintaining crop productivity under variable climatic conditions, and sustaining the livelihood and nutritional security of smallholder farmers (Keatinge et al., 2011; Foyer et al., 2016). Shah et al. (2003) reported that mungbean can fix up to 110 kg N ha⁻¹ which can be used to satisfy the N needs of the companion or subsequent crops. Li et al. (2009) found that intercropping mungbean with rice could increase the formation of mycorrhiza and nodules, thus increasing N and P uptake and N transfer to associated crops. The only reported mungbean study in intercropping systems in Senegal demonstrated that mungbean was a compatible legume crop in millet systems (Trail et al., 2016). Studies on the benefits of mungbean consumption in Senegal also reported agricultural advantages from its cultivation which improved dietary diversity of women and also provided financial opportunities (Vashro et al., 2018). With the projected increase of semi-arid areas in Senegal, the introduction of mungbean to Senegalese agriculture could help sustain agricultural production through diversification of agricultural systems. It also represents an opportunity to produce nutrient-rich foods in an area that faces serious food insecurity issues.

The primary goal of this proposed research is, therefore, to enhance the productivity of cropping systems and to improve soil fertility and food security in Senegal. Specifically, the objectives of the proposed experiments are: 1) to evaluate the effect of inoculation of mungbean with *Bradyrhizobium* (group I) on growth, yield, yield components of two mungbean genotypes in greenhouse studies; 2) evaluate the effect of millet and mungbean intercropping on the yield

of combined crops in Senegal; and 3) screen the best adapted lines for inherent N fixation capacity under favorable conditions in the greenhouse.

Dissertation structure

The dissertation begins with the rationale and significance of mungbean (Chapter 1) as a highly productive legume crop in N-impooverished soils and under highly variable climatic conditions. Chapter 2 reviews the potential of mungbean for diversity cropping systems and improving soil fertility in SSA. On the basis of the great potential of mungbean for production in SSA for restoring soil fertility and nutritional security, a greenhouse study (Chapter 3) was conducted to investigate the effects of *Bradyrhizobium* (group I) inoculation and soil texture on mungbean establishment, yield and yield components. To assess the effects of mungbean association with millet systems (Chapter 4), field-based research trials were conducted in Senegal. The results showed that the successful inclusion of mungbean in cropping systems can improve household food security and dietary diversity for smallholders. As a legume crop, mungbean is capable of fixing atmospheric N₂ for its own N requirements and the N needs of the companion crops. Thus, mungbean genotypes were used to estimate biological N₂ fixation using the ¹⁵N natural abundance method (Chapter 5). Overall, this research project proposes a legume diversification of cropping systems which provides economically sustainable advantages for farming through biological nitrogen fixation in Sub-Saharan Africa.

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Chapter 2 – Potential of mungbean [*Vigna radiata* (L.) Wilczek] in Sub-Saharan Africa. A review

Abstract

Mungbean [*Vigna radiata* (L.) Wilczek] is an important tropical legume mainly cultivated in South and East Asia. Despite being a minor grain legume in Sub-Saharan Africa (SSA), mungbean has a considerable potential for improving soil fertility and enhancing food security of smallholder farmers. Mungbean is a short-duration and drought-tolerant crop, capable of symbiotic atmospheric nitrogen fixation. It has low water and input requirements, making it suitable to rainfed smallholder production systems. Having similar nutritional content to cowpea and dry beans, mungbean could perform better than them under semi-arid conditions due its lower rate of flower and pod abscission. The legume is an important source of protein, carbohydrates, minerals (particularly iron), and vitamins and has lower phytic acid content than other legumes and staple cereals in SSA. Mungbean seeds can be eaten with cereals, processed to make dhals, sprouts, noodles, soups, desserts, and protein- and iron-rich supplements for children. This review summarizes (1) the advantages of mungbean cultivation and (2) its potential for improved nutrition in SSA as well as (3) constraints, highlighting (4) the potential characteristics for mungbean improvement in SSA.

Keywords: Mungbean, Sub-Saharan Africa, soil fertility, food security.

Introduction

In Sub-Saharan Africa (SSA), the projected increase in climate variability, as well as increased frequency and intensity of extreme weather events, is expected to have a negative impact on agricultural production (Ogallo, 1984; Kotir, 2011; Niang et al., 2014). Rainfall variability and

persistent droughts and floods are reported to contribute to decreased crop yields (Lloyd et al., 2011), fluctuation and volatility of food prices (Terdoos and Feola, 2016), negative impacts on livelihoods (Connolly-Boutin and Smit, 2016; Diatta et al., 2016a), and increased poverty and malnutrition (Barrett and Bevis, 2015; Tirado et al., 2015). Adaptation strategies must occur within the context of both sustainable agricultural production and growing consumer demand.

Development of climate adaptation strategies and mitigation efforts that would anticipate effective responses and interventions will be important in Sub-Saharan Africa where are located the most vulnerable populations (Kotir, 2011; Niang et al., 2014; Zewdie, 2014). Such strategies include improvement of agricultural management practices (Brooker et al., 2015) and sustainable intensification (Lal et al., 2015; Snapp et al., 2019), use of high-yielding (Brown et al., 2009; Cairns et al., 2013) and drought and heat-resistant crop genotypes (Odeny, 2007; Ramírez Villegas and Thornton, 2015), intensified use of technology inputs (Shishaye, 2015), natural resource stewardship (Mbow et al., 2008; Diatta et al., 2016b; Faye et al., 2019), and development of policy and community programs (Connolly-Boutin and Smit, 2016). Growing mungbean, a species with low water and input requirements and wide adaptability into crop rotations, is a potentially promising way of increasing crop production under adverse soil, water, and climatic conditions. Furthermore, the high protein, mineral, and vitamin content of mungbean represent an opportunity for improving nutrition security in SSA. However, while mungbean has played an important role in improving soil fertility and sustaining the livelihood and nutritional security of smallholder farmers in other regions (Smith et al., 2016; Ullah et al., 2016), it represents an understudied crop in SSA (Naylor et al., 2004; Foyer et al., 2016).

Mungbean is an important tropical food legume that has an optimum temperature range for growth of 28-30°C and is extensively grown in areas that are prone to drought and high

temperatures (Sangakkara et al., 2001; Kumar and Kumar, 2014; Raina et al., 2016). Global annual production of mungbean in 2017 was estimated to be approximately 2.7 mill metric tons, which represented about 3% of global pulse production that year (IMARC, 2018). Mungbean global production is expected to be 5.3 million metric tons by 2023, representing a 15% increase due to an increased demand from the food industry (IMARC, 2018).

Mungbean is a self-pollinated diploid ($2n = 22$), erect plant with branches carrying pods (8-15 seed grains) in clusters near the top of the plant (Kaur et al., 2015), belonging to the *Papilionoideae* in the *Fabaceae* (Van et al., 2013). It belongs to the *Phaseoleae* tribe, which contains soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) (Stefanović et al., 2009). The genus *Vigna* includes more than 100 species in three sub-specific taxa: *radiata* (green grams and golden grams including the cultivated mungbean), *sublobata* and *glabra* (Van et al., 2013; Sakai et al., 2015). Improved mungbean varieties reach maturity in 60 to 65 days (Fig. 1) (Keatinge et al., 2011) while traditional varieties mature in 65 to 90 days (HanumanthaRao et al., 2016). Mungbean varieties have determinate growth but flower and fruit over a period of several weeks, which can result in multiple harvests (Fig. 1) (Diatta et al., 2018). In semi-arid regions, mungbean grain yields exceeding 1.5 Mg ha^{-1} have been reported for improved varieties (Chadha, 2001; Mbeyagala et al., 2017; Kassa et al., 2018), while traditional varieties average about 0.5 Mg ha^{-1} (Mogotsi, 2006; Nair et al., 2012). Mungbean is classified by the Food and Agriculture Organization (FAO), as a “dry bean”. Few studies have been conducted to assess the adaptation, growth, and yield of introduced mungbean genotypes in Africa. No prior article has reviewed the potential of mungbean in Sub-Saharan Africa to our knowledge.

Geographic distribution and production of mungbean

Genetic diversity data and archaeological studies revealed mungbean to be a native crop of India and the Indo-Burma region where the early domestication processes began (Morton et al., 1982). From this region, selection of species resulted in the introduction of cultivated types of mungbean by Asian emigrants or by traders to the Middle East, Africa, Latin and South America, and Australia (Smartt, 1984). *Vigna radiata* var. *sublobata* is believed to be the wild progenitor of mungbean.

Mungbean is cultivated on more than 6 million ha (HanumanthaRao et al., 2016). Ninety percent of global production occurs in south, east, and southeast Asia (Kang et al., 2014). India represents the major legume producing country in south Asia and the largest producer of mungbean worldwide followed by China and Myanmar. India's annual mungbean production is estimated to be around 3 million metric tons, which represents over 50% of the total world production (Nair et al., 2015). Because of both the limited increase in mungbean production over the past years combined with limited access to high quality seeds, current demand is high in India. Myanmar (83%), Tanzania (4%), Kenya (4%), Australia (3%), and Mozambique (2%) export mungbean to India (Raina et al., 2016).



Figure 1. A guide to the growth stages and agronomic advantages of mungbean.

In Sub-Saharan Africa, legume production is dominated by peanut, cowpea, and dry bean (Fig. 2). However, limitations on these primary legume crops in coping with drought provide an opportunity for mungbean production. In Africa, mungbean is cultivated in at least 22 countries but still remains as a minor crop in the continent, except in Kenya where it represents a valuable source of protein, nutrients and income to rural communities (Mogotsi, 2006). Among eight pulses tracked by FAOSTAT, aggregate production in SSA doubled to 30 million metric tons in the 21 years from 1997 to 2017. Peanut production grew only a little, but most of the other pulses more than doubled production (Fig. 2).

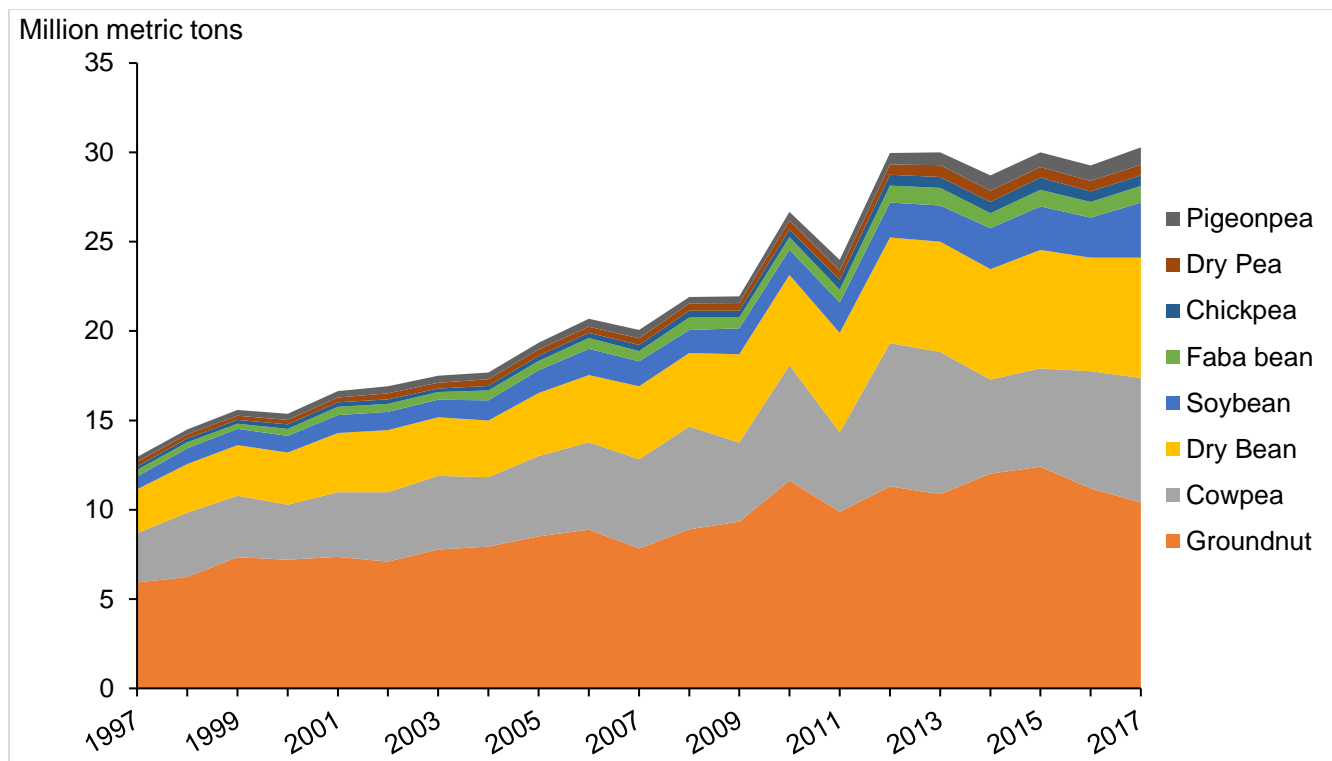


Figure 2. Annual production of major pulse crops in Sub-Saharan Africa during the period between 1997 and 2017 (Mill metric tons). *Source:* FAOSTAT (2019)

While production of major legume crops such as groundnut, cowpea and common beans has seen an important increase in Sub-Saharan Africa in the last 20 years, mungbean production has remained low and stable (Keatinge et al., 2011). One of the reasons behind this phenomenon could be explained by the low adoption of superior genetics by farmers in SSA. In major mungbean-producing countries in this region, recent reported average yields fluctuated between 0.2 to 0.5 Mg ha⁻¹ (Waniale et al., 2014) while potential yields of 2.0 Mg ha⁻¹ have been reported in trials (Herridge et al., 2005). Although use of improved mungbean lines developed by The World Vegetable Center have shown promising results in Asia, African farmers still use traditional varieties that are low-yielding, small-seeded, and pod shattering (Waniale et al., 2014). A participatory selection study of mungbean genotypes conducted in Uganda has revealed farmers'

preferences for high-yielding and large-seeded genotypes compared to landraces (Mbeyagala et al., 2017). These findings suggest that breeding efforts to enhance mungbean productivity need to focus on farmers' needs in addition to genotypes with superior traits (Mbeyagala et al., 2016).

Agronomic performance of mungbean

Mungbean is usually grown on marginal lands under rainfed conditions. It is well adapted to arid and semiarid conditions and is suitable for planting on a range of altitudes, temperatures, and soil types. However, it grows best in subtropical regions with average annual rainfall in the range 600-900 mm and at altitude not exceeding 2000 m on well-drained loams or sandy loam soils (Mogotsi, 2006). Mungbean is well-suited to many cropping systems due to its ability to improve soil fertility and sustain productivity of subsequent crops in subsistence agriculture (Senaratne et al., 1995; Dey et al., 2016). In Sub-Saharan Africa, where reside the world's most vulnerable food production systems, recurrent droughts are projected to increase in frequency and intensity (Kotir, 2011; Zewdie, 2014; Connolly-Boutin and Smit, 2016). As a result of this, 60 to 90 million ha could be transformed into new arid and semi-arid areas in the following years, threatening food security for local farmers (UNDP, 2007; Juana et al., 2013; Rao et al., 2019). Under such a scenario, a highly nutritious crop like mungbean, which can sustain high rates of productivity under moderate to accentuated drought conditions, deserves special consideration. Multiple factors contribute to mungbean's drought-tolerance, such as early seedling growth stage (significantly positive correlation ($P < 0.05$) with drought-tolerance (Zhongxiao et al., 2012)), important overall root system mass and length of main root, efficient stomatal conductance, and good photosynthetic capacity under water stress (Oo et al., 2005). Mungbean also has a low rate of flowers or pods abortion (14-37% compared to 43-81%, 48-76%, and 70-88% in soybean, common bean, and cowpea, respectively) (Ojehomon, 1968; Avav and Ugehe, 2009).

Mungbean is an environmentally sustainable food legume, maintaining or enhancing soil fertility and reducing inorganic nitrogen needs. However, application of nitrogen fertilizer has been reported to improve mungbean growth and yield under low soil fertility conditions (Yin et al., 2018). To assess the effects of six nitrogen fertilizer rates (0, 20, 40, 60, 80 and 100 kg N ha⁻¹) on growth and yield of mungbean, Razzaque et al. (2017) conducted a pot study in a low-nutrient environment. They reported that 60 kg N ha⁻¹ recorded 14.2 g plant⁻¹, which was a 48% increase over control. Under field conditions, Khan et al. (2008) investigated the effects 0, 30, 60, and 90 kg ha⁻¹ of N on yield and yield components of mungbean and found that N fertilizers significantly increased N-content of nodules and shoots, number of pods and yield (11%, 9%, and 9% increase) compared to control. They also performed an economic analysis on N fertilizers vs control which revealed that N applications gave net higher income ha⁻¹ than control under these conditions.

Like other legumes crops, seed inoculation of mungbean with an appropriate strain of *Rhizobium* spp. can result in a 10-12% increase in productivity (Ali and Gupta, 2012; Diatta et al., 2018). In addition, arbuscular mycorrhizal fungi have increased the productivity of mungbean and other legumes. Mycorrhizal dependence of blackgram, bushbean, chickpea, cowpea, gardenpea, grasspea, lentil, and mungbean revealed a dependence ranging from 25 to 51% for legume crops (Molla et al., 2011). This symbiotic relationship between mungbean, *Rhizobium* ssp. and arbuscular mycorrhizal fungi can promote and sustain mungbean productivity, even under drought conditions (Song, 2005; Habibzadeh et al., 2013). Arbuscular mycorrhizal fungi, with a 50% dependence recorded in mungbean (Molla et al., 2011), improves the uptake of slowly diffusing soil nutrients like phosphorus and micronutrients such as zinc (Tajini et al., 2011).

Intercropping systems are common agronomic techniques extensively practiced by subsistence farmers in Sub-Saharan Africa (Matusso et al., 2014; Corbeels et al., 2019).

Intercropping cereals with legume crops such as mungbean could increase the productivity of the land and also minimize the risk of crop failure (Brooker et al., 2015; Sabbagh and Lakzayi, 2016; Moswetsi et al., 2017). In Senegal, Trail et al. (2016) intercropped pearl millet [*Pennisetum glaucum* (L.) R. Br.] with mungbean and noted a 36% increase in millet grain yield compared to millet alone. Roy et al. (2016) reported that intercropping systems of maize and mungbean resulted in greater growth and higher number of cobs per plant, and grains per cob for maize, and pods per plant and seeds per pod for mungbean. High yield was attributed to a lower competition for light, water, and nutrients in these systems. Shaker-Koochi et al. (2014) intercropped mungbean with sorghum in a field experiment conducted in Iran and reported that intercropping treatments had higher intercropping advantage (3.22), relative yield totals (1.36) and land equivalent ratio (>1) compared to monocropping.

From an environmental standpoint, mungbean can improve agricultural systems in Sub-Saharan Africa through *in-situ* moisture conservation and addition of nutrient and organic matter to soil when grown as a green manure and/or cover crops (Morton et al., 1982; Samal et al., 2017). Among others, characteristics such as early establishment, high seedling vigor and N₂ fixation efficiency, short growing season with significant biomass production, favorable nitrogen to carbon balance, and easy incorporation and quick degradation into the soil, make mungbean a suitable crop for soil improvement in the Sub-Saharan Africa (Peoples and Herridge, 1990). Using mungbean as cover crop in semi-arid watersheds has resulted in reduced soil erosion through a decrease of runoff (28%) and sediment (30%) losses compared to bare soil (Rashid et al., 2015). Using mungbean as a cover crop may also reduce weed infestation in the companion crop (Dwivedi et al., 2016). Weed density and fresh weed biomass were reduced by 34% and 54%, respectively, when maize was intercropped with mungbean (6:10 row ratio) compared to maize alone in

Pakistan (Shahida and Khan, 2016). Planting mungbean at 35 cm row spacing + hand weeding at 25 days after sowing also reduced weed density and fresh weed biomass by 41 and 79%, respectively, compared to control (Awan et al., 2009). Moreover, mungbean has been used as an environmentally sound and sustainable approach for managing insects in cropping systems. A study conducted by Lu et al. (2009) to assess the potential of mungbean as trap crop revealed a 50% decrease in mirid bug [*Apolygus lucorum* (Meyer-Dür) (Heteroptera: Miridae)] population densities compared to cotton fields without mungbean plants (36 individuals per 100 plants). Similar findings were also reported by Geng et al. (2012) who observed not only significantly higher number of adults and first instar nymphs of *A. lucorum* but also higher adult longevity and fecundity on mungbean compared to cotton plants ($P < 0.05$). To understand the migration of *A. lucorum* adults between neighboring cotton and mungbean fields, Wang et al. (2017) developed a DNA-based polymerase chain reaction (PCR) approach. Findings from this study revealed a detection of cotton DNA in the guts of *A. lucorum* collected from mungbean plots evidencing the migration *A. lucorum* from cotton to mungbean plots. Results from these studies could help in developing mungbean-based trap-cropping strategies for controlling *A. lucorum* on agricultural crops.

Mungbean is also highly efficient in the use of nutrients, especially nitrogen, allowing smallholder farmers in Sub-Saharan Africa to achieve acceptable grain yields on marginal lands and under low fertility management. While mungbean crop has been successfully grown in SSA under low technology schemes to date, ensuring a timely and efficient nitrogen availability to the crop will represent a key management decision to increase grain yields in a region where little to none could be done regarding water availability to the crop. Nitrogen is the most essential nutrient and is a major component of protein, amino acids, enzymes, phospholipids, and nucleic acids. It

promotes plant growth and enhances the production of biomass and grain yield of legumes (Mian and Hossain, 2014; Carranca et al., 2015; Bibi et al., 2016; Fu and Shen, 2016). In the case of mungbean, field and lab research has demonstrated that, in symbiosis with *Rhizobium*, the crop can derive up to 75% of its total N requirement from N₂ fixation (Shah et al., 2003), fix up to 110 kg N ha⁻¹ (Dakora et al., 2015), and transfer 10% of fixed N to companion or following crops (Zang et al., 2015). However, studies within the SSA measuring N₂ fixation by mungbean have not been reported.

Estimated values of biological N₂ fixation for common legume crops in SSA reveal large variations in amount of N fixed (Fig. 3). In south, east, and southeast Asia, measurements of N₂ fixation revealed large variation (Table 1). In intercropping studies with maize and mungbean in Sri Lanka, Senaratne et al. (1995) reported that mungbean has the ability to fix up to 197 mg N plant⁻¹ which represents a 7 to 11% of N transfer to maize in an intercropped system. In Pakistan, Hayat et al. (2008) reported values ranging between 19 and 47 kg N ha⁻¹, which represented between 32% to 49% of the total N requirement of the crop across the whole growing season. However, a study conducted by Shah et al. (2003) in Pakistan revealed that a higher percentage of the total N requirement of the crop may be derived from N₂ fixation (i.e., 54 to 82%, or 55 to 86 kg N ha⁻¹). In the Philippines, George et al. (1995) reported that N₂ fixation by mungbean ranged from 61 to 90 kg N ha⁻¹ while Rosales et al. (1995) found N fixation values ranging from 21 to 85 kg N ha⁻¹. In other cases, data obtained from field experiments was less variable. Peoples et al. (1991) investigated N fixation using the ¹⁵N natural abundance method in Thailand and documented N fixation rates ranging between 64 to 66 kg N ha⁻¹, which represented around 90% of the total N requirement of the crop in this study.

Table 1. Estimates of fixed N by mungbean in field trials.

| Country | N fixed kg N ha ⁻¹ | N method used† | Reference |
|-------------|-------------------------------|-----------------------------------|-----------------------------|
| Pakistan | 55–86 | N balance | Shah et al. (2003) |
| Pakistan | 35–83 | ¹⁵ N isotope dilution | Mohammad et al. (2010) |
| Philippines | 25–47 | ¹⁵ N isotope dilution | Delfin et al. (2008) |
| Philippines | 21–85 | ¹⁵ N isotope dilution | Rosales et al. (1998) |
| Philippines | 61–90 | ¹⁵ N isotope dilution | George et al. (1995) |
| Philippines | 21–85 | ¹⁵ N isotope dilution | Rosales et al. (1995) |
| Thailand | 35–50 | ¹⁵ N isotope dilution | Phoomthaisong et al. (2003) |
| Thailand | 10 | ¹⁵ N isotope dilution | Toomsan et al. (2000) |
| Australia | 20–83 | ¹⁵ N natural abundance | Rochester et al. (1998) |
| Ethiopia | 8–25 | ¹⁵ N natural abundance | Raji et al. (2019) |
| Pakistan | 32–46 | ¹⁵ N natural abundance | Umair et al. (2011) |
| Pakistan | 41 | ¹⁵ N natural abundance | Hayat and Ali (2004) |
| Thailand | 64–66 | ¹⁵ N natural abundance | Peoples et al. (1991) |
| Pakistan | 6–32 | Ureide | Ali et al. (2013b) |
| Pakistan | 17–47 | Ureide | Hayat and Ali (2010) |
| Pakistan | 55 | Ureide | Hayat and Ali (2004) |
| Pakistan | 19–47 | Ureide | Hayat et al. (2008) |
| Pakistan | 13–26 | Ureide | Tariq et al. (2007) |

† See Unkovich et al. (2008) for description of techniques for measuring biological N₂ fixation.

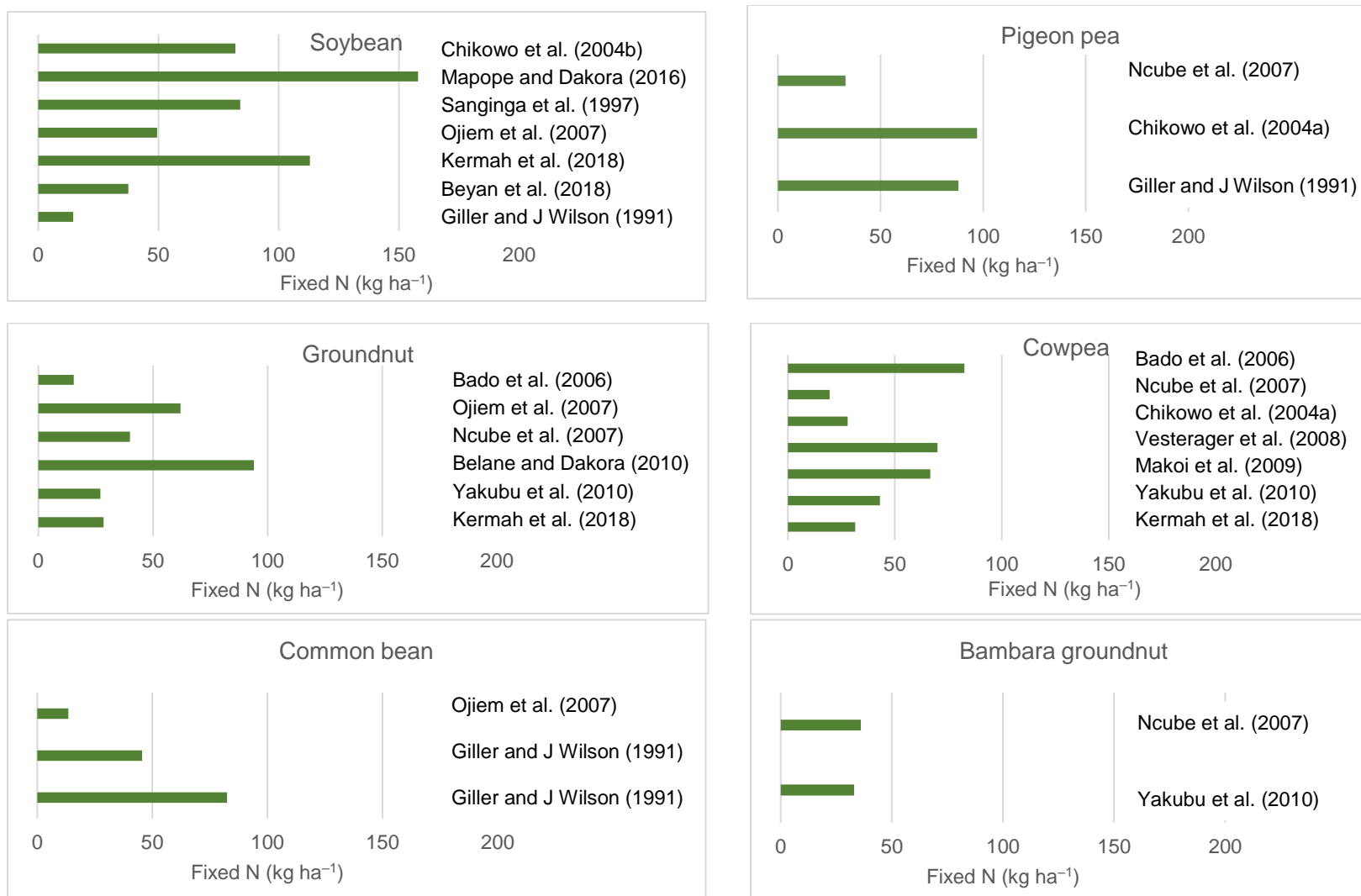


Figure 3. Estimates of fixed N (kg ha⁻¹) for some legume crops commonly grown in Sub-Saharan Africa.

Nutritional value of mungbean

The high nutritional value of mungbean makes it a good source of protein, minerals, and vitamins to smallholder households. Mungbean has a high protein content, complementing to deficiencies of cereal-based diets in SSA (Keatinge et al., 2011). Studies determining the proximate composition of mungbean report a wide variation in protein values (15% to 33%) (Paul et al., 2011; Shaheen et al., 2012; Dahiya et al., 2015; Ganesan and Xu, 2017). Mungbean has a comparable protein content to chickpea, kidney bean, cowpea, groundnut, and pigeon pea (Table 2) (USDA, 2010). Although mungbean is an important source of protein, its protein nutritional quality is limited by low concentration of sulfur-containing amino acids such as methionine and cysteine with 0.29 g and 0.21g in 100 g of raw edible portion (Nair et al., 2012; Ganesan and Xu, 2017).

Table 2. Absolute nutritional content (in g or mg) of major crop legumes grown in Africa and Asia. †

| Crop | Protein (g) | Oil (g) | Calcium (mg) | Iron (mg) | Zinc (mg) | Vitamin A (mcg-RAE) | Vitamin C (mg) | Folate (mcg) |
|-----------------|----------------|------------|-----------------|--------------|--------------|------------------------|-------------------|-----------------|
| Chickpea | 22 | 7 | 119 | 7 | 4 | 3 | 5 | 630 |
| Cowpea | 27 | 2 | 96 | 11 | 7 | 2 | 2 | 718 |
| Groundnut | 28 | 53 | 98 | 5 | 3 | 0 | 0 | 257 |
| Kidney bean | 27 | 1 | 162 | 9 | 3 | 0 | 5 | 446 |
| Mungbean | 26 | 1 | 145 | 7 | 3 | 7 | 5 | 687 |
| Mungbean sprout | 32 | 2 | 135 | 9 | 4 | 10 | 138 | 635 |
| Pigeon pea | 21 | 5 | 123 | 5 | 3 | 9 | 114 | 507 |
| Soybean | 40 | 22 | 303 | 17 | 5 | 1 | 7 | 410 |
| Soybean, green | 40 | 21 | 606 | 11 | 3 | 28 | 89 | 508 |

† Value per 100 g raw product (dry weight basis).

Source: (USDA, 2010)

Anti-nutritional compounds reduce the nutritive value of food due to limited digestibility, bioavailability, and bioconversion of nutrients. Anti-nutritional compounds reported in mungbean include tannins, phytic acid, hemagglutinins, polyphenols, trypsin inhibitor, and proteinase inhibitor (Dahiya et al., 2015). However, the reported amount of anti-nutritional components in mungbean like trypsin, hemagglutination, saponins, pythic acid and insoluble dietary fiber, are been relatively low compared to other legume crops such as soybean and cowpea (Elkowicz and Sosulski, 1982; Gupta, 1987) (Table 3). Variation in the amount of anti-nutritional components in mungbean can likely be explained by differences in genetic variation among cultivars (Dhole and Reddy, 2015). Processing techniques to decrease the concentration of anti-nutritional factors in mungbean include breeding research, agronomic techniques, and food preparation processes such as sprouting, dehulling, soaking, germination, boiling and cooking (Mubarak, 2005; Hemalatha et al., 2007; Ganesan and Xu, 2017). Preparing mungbean seeds with vegetables lowers the concentrations of anti-nutritional factors such as those displayed in Table 3 (Kumar Dahiya et al., 2014). Split seeds consumed with rice are beneficial for children and elderly people.

Table 3. Anti-nutritional factors in eleven legume flours.

| Crop | Trypsin (TUI mg ⁻¹) | Hemagglutination activity (HU mg ⁻¹) | Saponin (HA g ⁻¹) | Phytic acid (mg g ⁻¹) | Insoluble fiber (%) |
|---------------|------------------------------------|---|----------------------------------|--------------------------------------|------------------------|
| Chickpea | 18.8 | 7.9 | 0.5 | 5.2 | 3.8 |
| Cowpea | 12.2 | 2 | 12.8 | 7.6 | 4.3 |
| Faba bean | 4.8 | 10.6 | 0 | 3.7 | 2.7 |
| Field pea | 7.6 | 15.1 | 0 | 7.4 | 3.2 |
| Lentil | 5.1 | 14.6 | 5.6 | 6.4 | 3.7 |
| Lima bean | 46.8 | 0.9 | 6 | 5.2 | 3.9 |
| Lupin | 0 | -- | 0.8 | 8.2 | 5.6 |
| Mungbean | 10 | 3.4 | 0.8 | 5.1 | 2.8 |
| Navy bean | 18.2 | 46.1 | 12.6 | 10 | 4.6 |
| Northern bean | 18.1 | 16.8 | 7.8 | 10.6 | 3.2 |
| Soybean | 41.6 | 77.4 | 0 | 11.6 | 5.9 |

Source: (Elkowicz and Sosulski, 1982)

Additionally, mungbean seeds are an important source of carbohydrates (59-65%), minerals (particularly iron), vitamins and amino acids in human diets (Dahiya et al., 2015) (Table 2). Minerals present in mungbean seeds include iron, calcium, phosphorous, magnesium, and potassium (Puranik et al., 2011). Mungbean seeds contain 1-1.5% fat, and 3.5-4.5% fiber, (Nair et al., 2013). Adding to its highly desirable nutritive composition, mungbean is also considered valuable for good health and human development because of the high digestibility of its protein and carbohydrates (Anwar et al., 2007). The digestibility value of mungbean (67-72%) is comparable to chickpea (65-79%), pigeonpea (60-74%), soybean (63-72%), and urd bean (56-63%) (Chitra et al., 1995). Mungbean is also a rich source of amino acids like arginine, isoleucine, leucine, lysine, phenylalanine, valine, aspartic acid, glutamic acid and serine (Table 4) (Dahiya et al., 2015). The relatively high protein and lysine content, added to the low content of methionine in mungbean makes it a good complement for cereals with high carbohydrate, low lysine and high methionine concentrations (Arvind et al., 2013).

Table 4. Amino acid composition of mungbean seeds.

| Amino acid (g/16 g of nitrogen) | Average† | Minimum | Maximum |
|---------------------------------|----------|---------|---------|
| Alanine | 4.1 | 3.6 | 4.5 |
| Arginine | 5.8 | 4.5 | 6.7 |
| Aspartic acid | 13 | 12 | 15.1 |
| Cysteic acid | 13.5 | 13.5 | 13.5 |
| Glutamic acid | 18.3 | 13.6 | 21.7 |
| Glycine | 3.6 | 3.2 | 4.3 |
| Histidine | 3.2 | 2.4 | 5.6 |
| Isoleucine | 4.3 | 3.6 | 5.4 |
| Leucine | 7.6 | 6.9 | 8.7 |
| Lysine | 6.5 | 4.1 | 8.1 |

| | | | |
|---------------|-----|-----|-----|
| Methionine | 1.2 | 0.5 | 1.9 |
| Phenylalanine | 5.4 | 4.6 | 6.2 |
| Proline | 4.5 | 3.7 | 5.6 |
| Serine | 4.9 | 4 | 5.8 |
| Threonine | 3.2 | 2.7 | 4 |
| Tryptophan | 1.2 | 0.5 | 3.4 |
| Tyrosine | 2.7 | 2.2 | 3.3 |
| Valine | 5.1 | 4.1 | 6.4 |

† Mean value of all collected data.

Source: Dahiya et al. (2015)

Food, feed and non-food uses of mungbean

Mungbean is an important food and livestock feed legume crop in tropical and subtropical regions and is extensively consumed for its protein-rich grains (Ramanujam, 1981; Bhardwaj et al., 1999; Khattak et al., 2007). Mungbean grains are typically consumed as boiled or cooked with vegetables or meat (Keatinge et al., 2011). It can also be used to make sprouts, soups, noodles, dessert and several other food products (Dhayal et al., 2015). In East Africa, mungbean is a major food crop and is commonly consumed as vegetable and processed seed. In Kenya and Tanzania, mungbean green pods and immature seeds are consumed with a popular thick maize porridge called “ugali” (Nair et al., 2013). Mature seeds of mungbean are also commonly boiled together with maize, sorghum and other cereals or fried with meat or vegetables in Kenya (Nair et al., 2013). In Uganda, mungbean represents an important food product and source of income for smallholder farmers (Waniale et al., 2014). Consumption of cooked mungbean seeds in sauces and as a side dish is common in Ethiopia and Malawi, respectively (Mogotsi, 2006). In West Africa, recent efforts to improve food security and soil fertility through crop diversification have resulted in the

development and introduction of mungbean (Ngwuta et al., 2010; Diatta et al., 2019a). In Nigeria, mungbean is consumed as sprouts in salad or processed into biscuits (Akaerue and Onwuka, 2005; Okweche and Avav, 2013). Mungbean seeds and leaves are boiled and consumed with rice or millet in Senegal (Abaye et al., 2018). A study on dietary diversity of women and children conducted in Senegal revealed that inclusion of mungbean into the Senegalese diet could be a major addition to limited legume consumption and supplement cereal-based diets (Vashro, 2017).

In India, mungbean is consumed as whole or split seeds which are transformed into a thick soup called “dhal” (Chadha, 2001). In China, food products made of mungbean include soup, porridge of mungbean and rice, sprouts, starch noodles, and cakes. Cold jellies and cakes represent the popular food products in Thailand (Nair et al., 2013). After removing the seed coat, mungbean seeds may also be ground into flour. Mungbean flour can be further transformed into various products such as noodles, bread, biscuits, and vegetable cheese, used to fortify wheat flour, or to formulate high-protein food supplements for children (Nair et al., 2013). Imtiaz et al. (2011) revealed that 44% wheat flour with 36% mungbean flour or 56% wheat flour with 24% mungbean flour combined with 10% skim milk powder and 10% sugar in both cases can be used as weaning food. However, work on the effects of processing methods on protein concentration has shown that processing could improve the nutrient composition of mungbean flours (Akaerue and Onwuka, 2010).

Mungbean may provide opportunities for improving the health of rural populations in Sub-Saharan Africa. The relatively high concentration of proteins, amino acids, oligosaccharides, and polyphenols in mungbean make it suitable for antioxidant, antimicrobial, anti-inflammatory and anti-tumor use (Tang et al., 2014). Mungbean soup has been successfully used to increase total antioxidant capacity and glutathione levels, and to subsequently alleviate heat stress in rats (Cao

et al., 2011). Results from the study demonstrated the potential of mungbean soup in reducing the risk of heat stress in humans.

Mungbean crop residues are a good quality forage for livestock, particularly as a high-protein supplement to produce high-quality meat and milk. Sherasia et al. (2017) reported that fresh forage mungbean contains 13-21% of protein on a dry matter basis and mungbean straw has 9-12% protein content. Forage yields of non-fertilized mungbean plants averaged 0.64 t ha⁻¹ while 1.4 t ha⁻¹ was recorded under fertilized conditions (Sherasia et al., 2017). However, aboveground samples of mung bean for forage yielded 2.9 t ha⁻¹ in the Southern Great Plains (Rao and Northup, 2009). Because mungbean matures quickly, it offers forage while other legume crops such as cowpea or velvet bean are still maturing (Lambrides and Godwin, 2007).

Major constraints to mungbean production

The productivity of mungbean in Sub-Saharan Africa, widely grown over a range of environments, is constrained by abiotic and biotic stresses (Tuba et al., 2010; HanumanthaRao et al., 2016). Among abiotic stresses, drought and flooding are two of the major constraints to mungbean production in SSA. High rainfall variability has led to a reduction in suitable lands for bean production and a subsequent decrease in agricultural production (Foyer et al., 2016). Drought and flooding stresses have been reported to limit growth and yield of mungbean (Lalinia et al., 2012; Amin et al., 2016). To understand the effects of water stress on phenological and agronomic traits of mungbean, Lalinia et al. (2012) applied four irrigation regimes (no water stress, drought stress at the flowering, during pod and seed formation) to five mungbean cultivars. They found that drought stress manifested in mungbean through decreased plant height, 100-grain weight, number of grains per pod, number of pods per plant, days to flowering and physiological maturity. This decrease in mungbean growth and yield components when grown under drought stress

conditions could be explained by inefficient stomatal regulation and low photosynthetic capacity under limited soil moisture stress conditions (Sunil et al., 2015; Raina et al., 2016). It could be also due to low xylem water potential, low transpiration resistance, high leaf diffusive resistance, and high canopy minus air temperature differential (Naresh et al., 2013). An excess of water can be detrimental to mungbean productivity. Working with five mungbean genotypes in Bangladesh, Amin et al. (2016) found that a 4-day flooding imposed at 24 days after emergence induced a decrease in total dry matter and seed yield through a reduction in the pods per plant and the seed size of all genotypes. The decrease in mungbean productivity could be explained by the reduction in leaf photosynthesis, stomatal closure, and growth inhibition (Oo et al., 2005).

Low productivity of mungbean could be explained by poor agronomic practices such as growing mungbean under low soil moisture and fertility conditions, absence of appropriate rhizobial strains, and inefficient weed control. Increases in mungbean productivity are often limited by an insufficient supply of suitable cultivars and high-quality seed (Keatinge et al., 2011; Foyer et al., 2016). Fewer pest and disease problems have been reported in mungbean compared to other legumes such as soybean, common bean and cowpea resulting in more stable yields (Keatinge et al., 2011).

Yield loss of mungbean can be caused by field pests such as whitefly, *Bemisia tabaci* Genn, leaf hopper, *Empoasca kerri* Pruthi, black aphid, *Aphis craccivora* Koch, Bihar hairy caterpillar, *Diacrisia obliqua* (WIK), galerucid beetle, *Madurasia obscurella* Jacoby, stem fly, *Ophiomyia (Melanagromyza) phaseoli* (Tryon), lycaenid borer, *Euchrysops cnezeus* Fabr, and spotted caterpillar, *Maruca testulalis* Geyer (Lal, 1985). Integrated management strategies of mungbean pests include resistant cultivars, clean seeds, cultural practices, and biological control and chemical pesticides (Swaminathan et al., 2012). The major viral disease that constrains

mungbean production is Mungbean Yellow Mosaic Virus (MYMV) (Anjum et al., 2010; Dubey and Birendra, 2013; Nair et al., 2019). MYMV is caused by *Begomovirus* species transmitted by whitefly (*Bemisia tabaci* Gennadius) (Sugandh et al., 2015). Mungbean plants infected by MYMV have small and scattered yellow colored leaves, reduced flower production, and small immature seeds in shrunken pods (Patel and Mahatma, 2016), resulting in a drastic reduction of mungbean yield from 30 to 100% (Kumar and Singh, 2008). Reduced yield can cause substantial economic losses (Dubey and Birendra, 2013). The prevalence of MYMV has been reported to be favored by high temperatures, high humidity (Safdar et al., 2015) and abundant whiteflies population (Munawwar et al., 2014). The development of high-yielding and disease-resistant mungbean varieties resulted in its introduction in rice/wheat rotations in Asia and a subsequent increase in crop production (Keatinge et al., 2011). Breeding efforts against MYMV also resulted in the development of high-yielding disease-resistant varieties (Kumar and Singh, 2008). Munawwar et al. (2014); Munawwar et al., 2014; Sugandh et al. (2015) identified mungbean genotypes resistant to MYMV through a screening trial. They reported that these genotypes could be used for development of MYMV-resistant varieties. However, Mohan et al. (2014) and (Munawwar et al., 2014) conducted a screening of mungbean genotypes for resistance to MYMV under field conditions and reported that most of the studied germplasms exhibited moderate to high susceptibility. Similarly, immune or highly resistant genotypes against MYMV under field conditions were not found by Binyamin et al. (2015) during a screening experiment. However, MYMV in mungbean has not been reported in SSA.

Additional major diseases of mungbean include powdery mildew [*Podosphaera fusca* (Fr.) U. Braun & Shishkoff], anthracnose [*Colletotrichum acutatum* (J.H. Simmonds)], cercospora leaf spots [*Cercospora canescens* Ellis & G. Martin], *Erysiphe polygoni* (Vaňha) Weltzien),

C. truncatum (Schwein.) Andrus & Moore, *C. gloeosporioides* (Penz.) Penz. & Sacc), and wet root rot [*Rhizoctonia solani* (Kuhn)] (Anjum et al., 2010; Dubey and Birendra, 2013; Nair et al., 2019). Options to reduce the impacts of mungbean pathogens involve integrated disease management such as combinations of insecticides, fungicides, and bio-formulation as seed treatment. Dubey and Birendra (2013) revealed that mungbean seeds treated with a combination of thiamethoxam (insecticide) at 4 g kg¹, carboxin (fungicide) at 2 g kg¹ and Pusa 5SD (*Trichoderma virens*) at 4 g kg¹ recorded a low incidence of cercospora leaf spots, MYMV, and wet root rot.

Lack of policy and research attention can also constrain the productivity of mungbean in Sub-Saharan Africa (Akibode, 2011). Increases in mungbean productivity are often limited by an insufficient supply of suitable cultivars and high-quality seed coupled with the lack of training programs on mungbean potential benefits for agricultural productivity, soil, and human health (Shanmugasundaram et al., 2010; Keatinge et al., 2011; Foyer et al., 2016). As a result, adaptive and strategic research associated with the development of strong network and financial support will be helpful to promote mungbean from being a marginal crop to become one of the major grain legume crops in SSA, as was the case in Asia (Nair et al., 2012).

Potential for mungbean improvement

Agronomic practices for improving mungbean yields include optimization of row spacing and plant density in order to increase mungbean production through increased cumulative intercepted radiation and increased water use efficiency for specific environments (Rachaputi et al., 2015). The available, limited mungbean literature for SSA highlights the importance of investigating the potential of mungbean production (Table 5). Diatta et al. (2018) reported that inoculation of mungbean with *Bradyrhizobium* inoculum (group I) increased the number of pods per plant, number of seeds per plant, and seed yield by 15%, 18%, and 14%, respectively over

uninoculated mungbean. HanumanthaRao et al. (2016) and Trail et al. (2016) suggested that surface organic mulch could also be used to alleviate heat stress of mungbean under semi-arid conditions through a decrease in soil temperature and reduced loss of soil water.

Promotion and utilization of mungbean in agriculture in Sub-Saharan Africa will require enhancement of production and nutritional value through breeding and better management practices under environmental and economic constraints (Nadarajan and Chaturvedi, 2010; Auti, 2012; Dahiya et al., 2015; HanumanthaRao et al., 2016). In order to fully utilize mungbean to increase agricultural resiliency in SSA, the changing climatic conditions will need concomitant screening of existing varieties and genetic improvements to develop high yielding varieties with short growing season, disease-resistant, and tolerant to waterlogging and salinity (Kumar and Kumar, 2014; Raina et al., 2016). To this point, efforts to develop improved varieties of mungbean with synchronous maturity, short growing season (60-75 days), higher yields ($>2 \text{ Mg ha}^{-1}$) under different biotic and abiotic stresses, and better nutritional composition across various environments were recently initiated in South Asia (Kumar and Kumar, 2014). Despite these efforts, limited data on the genome sequence for *Vigna* species in developing countries resulted in a limited advancement of the molecular breeding research in these regions, particularly in SSA (Kang et al., 2014). Participatory selection of high-yielding and nutrient-dense cultivars should be continued in Eastern Africa but also encouraged in other Sub-Saharan regions (Mbeyagala et al., 2017).

In addition, breeding efforts should include sequencing of mungbean genome from existing germplasm collection and development of markers for traits of interest such as high yield, reduced photoperiod sensitivity, and synchronous maturity (60-65 days) (Kang et al., 2014; Pataczek et al., 2018). The availability of the complete genome sequence of mungbean represents an opportunity to facilitate the construction of genetic maps and the identification of important agronomic traits

for crop improvement (Kim et al., 2015). Sustainability of mungbean in SSA will also require resistance to specific diseases such as cercospora leaf spot, powdery mildew, and MYMV and insects such as bruchids (*Callosobruchus* spp.), bean fly [*Ophiomyia phaseoli* (Tryon)], and pod borer (*Maruca* spp.). The development of high-yield, short-duration, and disease-resistant varieties could result in widespread introduction across SSA. Alongside with these efforts, the development of testing multi-environments and coordination of testing across the region will be helpful to determine the adaptability and stability of improved mungbean genotypes in SSA (Mbeyagala et al., 2016).

Because of the wide genetic variability of mineral composition in mungbean varieties, biofortification has a great potential for enhancing micronutrient concentrations, and thus its nutritional quality (Kumar and Kumar, 2014; Dahiya et al., 2015). For example, interspecific breeding of mungbean with black gram [*V. mungo* (L.) Hepper], a close relative of mungbean, could be used to increase the low concentration of the essential amino acid methionine (Nair et al., 2013). The establishment of seed production and distribution systems and creation of agronomic and market opportunities will be necessary for smallholder farmers (Keatinge et al., 2011). The development of training programs in mungbean production such as the organization of field days and demonstration trials needs to be promoted to sustain food production in SSA (Keatinge et al., 2011). These training programs should also take into account the diversity of agro-ecological conditions and socio-economic factors in SSA (Moswetsi et al., 2017). Finally, expansion of mungbean in SSA may also require adequate financial support from national research institutes, international organizations, and the private sector in order to sustain and capitalize research findings on best agronomic practices, breeding efforts and adaptation strategies of mungbean to current agriculture systems in SSA.

Table 5. Summary of mungbean studies, including reported grain yield ranges, conducted across Sub-Saharan Africa.

| Country | Region | Rainfall (mm) | Temperature (°C) | Soil type | Yield range (Mg ha ⁻¹) | Culture system | Fertilization | References |
|----------|---|-----------------|------------------|-----------------|------------------------------------|--|---|--------------------------|
| Ethiopia | North Shewa Zone Amhara Region | NA ^a | NA ^a | NA ^a | 0.68 to 1.54 | Participatory on-farm evaluation | NA ^a | Kassa et al. (2018) |
| Ethiopia | Semi-arid highlands (Northern Ethiopia) | 635 | 13 - 24.4 | Clay loam | 0.49 to 1.37 | Performance under deficit irrigation application | Diammonium phosphate at a rate of 18/46 N/P ₂ O ₅ (100 kg/ha) | Ambachew et al. (2014) |
| Nigeria | Guinea savanna zone | NA ^a | NA ^a | Sandy loam | 0.44 to 2.27 | Screening of cultivars | NA ^a | Okweche and Avav (2013) |
| Nigeria | Rain forest zone of Southern Nigeria | NA ^a | 22.3 - 31.5 | Sandy loam | 0.37 to 0.53 | Preliminary field assessment | No fertilizer | Agugo et al. (2010) |
| Nigeria | Sub-humid savanna of Nigeria. | NA ^a | NA ^a | NA ^a | 0.54 to 2.00 | Reproductive abscission and seed yield | NA ^a | Avav and Ugeese (2009) |
| Senegal | Central millet-peanut basin | 398-529 | NA ^a | Loamy sand | 0.25 to 0.65 | Intercropping with millet | No fertilizer | Trail et al. (2016) |
| Uganda | North Western Farmland- Wooded Savanna | 468 | 18.7 - 30.8 | Sandy | 0.14 to 0.40 | Multi-environment trials | No fertilizer | Mbeyagala et al. (2016) |
| | Northern Moist Farmlands | 545 | 17.8 - 29.8 | Sandy | 0.47 to 0.63 | | | |
| | Southern and Eastern Lake Kyoga Basin | 494 | 17.4 - 29.3 | Sandy loam | 0.52 to 0.73 | | | |
| | North Eastern Short Grass Plains | 325.5 | 17.2 - 29.6 | Sandy loam | 0.32 to 0.46 | | | |
| | Northern Moist Farmlands | 560.5 | 16.8 - 29.3 | Sandy | 0.24 to 0.46 | | | |
| | Southern and Eastern Lake Kyoga Basin | 551 | 18.0 - 29.8 | Loam | 0.28 to 0.53 | | | |

^a NA, not available.

Conclusion

This paper highlights the promising yet largely unexploited potential of mungbean for diversifying and increasing crop productivity, promoting sustainable adaptation strategies, and reducing food insecurity and poverty in Sub-Saharan Africa. Mungbean's symbiotic relationship with rhizobia, agronomic advantages and nutritional potential makes it a valuable crop for meeting the ever-increasing global need for food and nutritional security. Broad production and consumption of mungbean in SSA should be encouraged by an active promotion of both good agronomic practices and information about the nutritional value of mungbean for human health.

Nutritional and agronomic benefits should be also given research and development attention supported by a multidisciplinary approach. Existing germplasm needs to be extensively screened to find the best varieties for varied environments in SSA and acceptability for local cuisine. For this reason, 550 mungbean varieties from USDA and AVRDC are being screened for best agronomic and nutritional traits in Senegal. In addition, variety screening is relatively inexpensive and provides immediate resources for growers, thereby promoting adoption. Whereas screening for physical environmental factors may be transferrable to similar environments that are geographically distant, the biological factors need to be considered. Finding good varieties for the expected temperature/soil/water conditions, and then screening those best varieties to understand their vulnerabilities to expected pests/diseases should be initiated.

Upon identifying the suite of biological constraints, it will be important to set priorities for breeding programs focusing on incorporating disease resistance into the varieties identified as best adapted to the physical environment. Thus, innovative mungbean breeding and agronomic technologies can be utilized to develop new varieties with superior agronomic, adaptive, and nutritional traits suitable for current cropping systems in SSA. Increased production and adoption

of mungbean can support sustainable production and improve the livelihoods of smallholder farmers in SSA.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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Chapter 3 – Inoculation and soil texture effects on yield and yield components of mungbean

Abstract

Mungbean [*Vigna radiata* (L.) Wilczek] is a short-duration and relatively drought-tolerant crop grown predominantly in the tropics. This grain legume can improve soil fertility through biological nitrogen (N) fixation. To assess the effects of *Bradyrhizobium* (group I) inoculation on yield and yield attributes of mungbean, a greenhouse study was conducted during Fall 2016 with two mungbean cultivars ('Berken' and 'OK2000'), two inoculum treatments (inoculated and uninoculated), and two soil textures (loamy sand and silt loam). Pots were laid out in a completely randomized design and treatment combinations were replicated seven times. The main effects of cultivar and soil texture significantly ($P \leq 0.05$) affected mungbean seed weight and plant residue mass. Seed yield (13%), plant residue (22%), and protein content (6%) of OK2000 were significantly higher than Berken cultivar. A 31% seed yield and 40% plant residue increase were recorded on silt loam soil compared to loamy sand soil. Significant increase in plant height (18%) and number of pods per plant (21%) were also recorded when mungbean plants were grown on silt loam compared to loamy sand soil. *Bradyrhizobium* inoculation significantly increased the number of pods per plant, the number of seeds per plant, and seed yield. [*Cultivar* × *inoculation*] and [*cultivar* × *soil texture*] interactions had significant ($P \leq 0.05$) effects on number of seeds per pods and plant height, respectively. Understanding the agronomic practices and soil physical properties that may limit mungbean production could help in optimizing its establishment and growth in non-traditional growing areas.

Keywords: *Bradyrhizobium* inoculation, yield, soil texture, *Vigna radiata* L., yield

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek], also known as mung, moong, green gram, golden gram, chickasaw pea, oregon pea, and chop suey bean, is a pulse species widely grown in tropical and sub-tropical regions of the world (Nair et al., 2013). It is an ancient crop believed to be a native of India and is extensively cultivated in South, East, and Southeast Asia, as well as East Africa (Kim, Nair, J. Lee, & S.-H. Lee, 2015; Sharma, Priya, Bindumadhava, Nair, & Nayyar, 2016). Mungbean is an important food and livestock feed legume crop in these regions and is consumed for its protein-rich seeds (Dahiya et al., 2015; Foyer et al., 2016). Mungbean, a short growth duration legume (55-110 days), fixes atmospheric N through a symbiotic association with rhizobia living in its root nodules (Peoples & Herridge, 1990; Razzaque, Haque, Karim, & Solaiman, 2016). However, under unfavorable conditions such as very acidic and heavy-textured soils, or very hot, dry conditions, *Rhizobium* survival and crop establishment can be constrained, thereby limiting root nodulation and yield (Kirchhof & So, 1995; Rahmianna, Adisarwanto, Kirchhof, & So, 2000). Therefore, seed inoculation with a strain of *Rhizobium*, appropriate to the cultivar and soil texture is recommended for improving growth and yield of mungbean (Hussain et al., 2014; Sharma & Khanna, 2010).

Anjum, Ahmed, and Rauf (2006) reported that *Rhizobium* inoculation of mungbean seeds increased the number of pods per plant, number of seeds per plant, seed weight and seed yield over the control. *Rhizobium* inoculation also increased plant height, number of nodules per plant, number of pods per plant, number of seeds per pod and weight of seeds per plant, and N and phosphorous (P) accumulation compared to the control (Hussain et al., 2014). Molla, Solaiman, Jahiruddin, Mridha, and Khanam (2011) observed that inoculation of seed with *Rhizobium* produced a higher number of nodules per plant, plant height, number of pod per plant and seed

yield. Travlos and Karamanos (2006) compared the responses of marama bean [*Tylosema esculentum* (Burch.) A. Schreib.], a perennial tropical grain legume of southern Africa on four different soil textures. They reported significant restriction in marama bean growth under clay and clay loam soils compared to sandy clay loam, and sandy soils. Thomson, Siddique, Barr, and Wilson (1997) evaluated the growth and seed yields of six commercially grown and eight potential new grain legumes species on a sandy clay loam and silty clay loam. They observed that faba bean (*Vicia faba*) cv. Fiord and field pea (*Pisum sativum* L.) cv. Dundale had the highest grain yield compared to other legume species on fine-textured, neutral to alkaline soils.

Most previous studies have shown that the use of a short-duration legume crop such as mungbean could be useful for producing nutrient-rich foods in regions facing food insecurity issues. However, few studies have investigated the yield performance of mungbean cultivars as affected by *Bradyrhizobium* inoculants under various soil conditions. The present study evaluated the combined effects of *Bradyrhizobium* (group I) inoculation, cultivar, and soil texture on yield and yield components of mungbean under greenhouse conditions.

Materials and methods

Soil description

A Eunola loamy sand and a Guernsey silt loam soil from 0-15 cm depth were collected from Virginia Tech's Tidewater Agricultural Research and Extension Center in Suffolk, VA (36°39'50.9"N latitude, 76°44'01.0"W longitude) and Kentland Agricultural Research Farm in Blacksburg, VA (37°11'47.3"N latitude, 80°34'49.7"W longitude), respectively. The site in Suffolk had been under a corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) rotation while the one in Blacksburg was subjected to a continuous corn system. The presence of naturally occurring

symbiotic bacteria in these samples was not evaluated, but due to the lack of legumes in either of these rotations, the presence of high levels of native bacteria was deemed unlikely. The field capacity of the soil samples was determined as described by Gupta and Larson (1979). Soil pH and plant available Ca, Mg, P, and K and were determined as described by Maguire and Heckendorn (2011). Soil pH was determined using a 1:1 (vol/vol) soil-water mix. Phosphorus and potassium were determined via extraction with Mehlich 1 at a 1:1 solution:soil ratio, (Mehlich, 1976) filtered (Whatman #2), and analyzed via inductively coupled plasma atomic emission spectroscopy (ICP) (SPECTRO ARCOS, Kleve, Germany). Total C and N were analyzed by dry combustion using a vario MAX CNS Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Prior to determining the concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) using Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography, the soil samples were prepared as described by Bremner and Keeney (1966). The physical and chemical properties of the two soil types are presented in Table 1.

Table 1. Chemical and physical characteristics of the Eunola soil and Guernsey soil

| Soil properties | Eunola soil | Guernsey soil |
|----------------------------|-------------|---------------|
| Soil texture | Loamy sand | Silt loam |
| Field Capacity (%) | 9 | 17 |
| Soil pH | 5.9 | 6.2 |
| C (%) | 0.98 | 0.93 |
| N (%) | 0.08 | 0.08 |
| C/N | 13 | 11 |
| NO_3^- (mg/kg) | 1.52 | 2.67 |
| NH_4^+ (mg/kg) | 0.32 | 0.25 |
| P plant available (mg/kg) | 37 | 25.67 |
| K plant available (mg/kg) | 62.67 | 61.33 |
| Ca plant available (mg/kg) | 575 | 858.67 |
| Mg plant available (mg/kg) | 72 | 167 |

Note. pH: soil (1:1 soil: deionized water mixture on a volumetric basis); Total carbon (C) and total nitrogen (N): vario MAX CNS Element Analyzer; Inorganic NO₃⁻ and NH₄⁺: 2M KCl extraction; Plant available nutrients: soil (Mehlich 1 solution).

Experimental design and management

A greenhouse study was conducted at a Virginia Tech greenhouse in Blacksburg (Virginia, USA) to examine the yield responses of mungbean cultivars to *Bradyrhizobium* inoculation and soil type. The experiment consisted of two mungbean cultivars, ‘Berken’ and ‘OK2000’, two inoculum treatments (inoculated and uninoculated conditions), and two soil textures (loamy sand and silt loam). The treatments were a factorial combination of three factors and the experiment was laid out as a completely randomized design with seven replications (Table 2).

Table 2. Cultivars, inoculation treatments, and soil texture used in the pot experiment

| No. | Treatments | | |
|-----|------------|--------------|--------------|
| | Cultivars | Inoculation | Soil Texture |
| 1. | Berken | Inoculated | Loamy sand |
| 2. | Berken | Inoculated | Silt loam |
| 3. | Berken | Uninoculated | Loamy sand |
| 4. | Berken | Uninoculated | Silt loam |
| 5. | OK2000 | Inoculated | Loamy sand |
| 6. | OK2000 | Inoculated | Silt loam |
| 7. | OK2000 | Uninoculated | Loamy sand |
| 8. | OK2000 | Uninoculated | Silt loam |

Bradyrhizobium inoculum (group I) purchased from Hancock Farm & Seed Co., Inc. (Dade City, FL, USA) was mixed with mungbean seeds at a rate of 10 g inoculant kg⁻¹ seed with to allow thorough coating and sown in the pots. The seed inoculant contained less than 1% active ingredient by weight of *Bradyrhizobium* sp. (*Vigna*), *Bradyrhizobium japonicum*, *Rhizobium leguminosarum* biovar *phaseoli*, *Rhizobium leguminosarum* biovar *viceae*. Mungbean cultivars were obtained from Oklahoma Foundation Seed Stocks (Stillwater, OK, USA). Berken is a medium-large seeded

cultivar while OK2000 is a large-seeded cultivar with good lodging and shattering resistance. Berken is widely grown in the U.S. and has 15% lower seed weight than OK2000 with similar seed yields. Experimental pots (19 cm tall, 19 cm outside diameter, and 3785 cm³ volume) were lined with polythene bags to avoid loss of water and filled with 4 kg of soil. Three seeds were sown (immediately after inoculation if applicable) per pot by hand at 3-4 cm depth on December 9, 2016 and grown at 80% field capacity. After seedling emergence, density was thinned to one plant per pot. Plant height was measured with a ruler at harvest maturity (March 14, 2017) by stretching out the plant to the tip. At full maturity, mungbean pods were hand harvested twice and the number of pods per plant and number of seeds per pod were counted. Seed yield was calculated as dry matter of seeds from each plant at harvest and seed yield per plant was computed. For plant dry matter determination, aboveground plant material was collected after seed harvest and oven dried at 50 °C to constant weight. Seed protein content was estimated using near infrared spectroscopy.

Analysis of variance (ANOVA) was used to test the effects of cultivar, *Bradyrhizobium* inoculation, and soil texture on plant parameters, seed yield and yield components using JMP Pro version 13.0.0 statistical software (SAS Institute Inc., Carey, NC). Differences among means were considered significant at the $\alpha = 0.05$ level of probability and were separated using Fisher's protected LSD. Most interactions were not significantly different for yield and yield components of mungbean, therefore most main effects were subjected to mean separation tests.

Results and Discussion

Plant height at maturity

The comparison of loamy sand with silt loam revealed that the average plant height (28.7 cm) of plants grown in silt loam soil was 18% greater than average height of plants grown in loamy

sand soil (Figure 1). These results are in agreement with Singh et al. (2011) who reported differences on mungbean growth when grown in different soil textures. They observed greater plant height on a sandy loam soil compared to a loamy sand soil and attributed this to higher organic carbon and moisture content in the sandy loam. Results obtained by Ntukamazina et al. (2017) revealed that differences in mungbean growth under different soils may be due to variations in moisture retention of soils. Increased height of plants grown in silt loam soils can likely be explained by better tilth compared to the sandy loam soil. Previous findings have demonstrated that different soil structures can result in differences in particle composition, chemical properties, mechanical impedance and bulk density which affect crop growth and development (Cook, Gupta, Woodhead, & Larson, 1995; Travlos & Karamanos, 2006).

Table 3. Analysis of variance of the effects of *Bradyrhizobium* inoculation, cultivar, and soil texture and their interactions on yield and yield components of mungbean (n = 7 per treatment)

| Treatments | Plant height ---- cm ---- | No. pods ----- plant ⁻¹ ----- | No. seeds ----- | Seed weight ----- g plant ⁻¹ ----- | Plant residue ----- | Protein -- % -- |
|-----------------------------------|------------------------------|---|--------------------|--|------------------------|--------------------|
| | <i>Pr > f</i> | | | | | |
| Cultivar | <.0001 | 0.8028 | 0.6637 | 0.0029 | <.0001 | 0.0003 |
| Inoculation | 0.2831 | 0.0029 | 0.0032 | 0.0013 | 0.0583 | 0.3178 |
| Soil Texture | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.3520 |
| Cultivar*Inoculation | 0.0247 | 0.3838 | 0.5031 | 0.8632 | 0.3995 | 0.6197 |
| Cultivar*Soil Texture | 0.8587 | 0.0657 | 0.0066 | 0.1036 | 0.8453 | 0.6134 |
| Inoculation*Soil Texture | 0.3554 | 0.6178 | 0.3362 | 0.2497 | 0.3107 | 0.7109 |
| Cultivar*Inoculation*Soil Texture | 0.7590 | 0.1384 | 0.0501 | 0.0672 | 0.6806 | 0.9616 |

The cultivar and inoculation interaction was statistically significant for plant height (Table 3). Height of inoculated OK2000 plants was greater than uninoculated OK2000 plants (30.0 vs. 26.8 cm, respectively) while inoculated and uninoculated Berken plants did not differ from each other

(mean = 23.9 cm) (Figure 2). Similar results were reported by Bhuiyan, Mian, and Islam (2008) who observed greater plant height for inoculated BARI Mung-2 cultivar due to increased nodulation resulting in enhanced vegetative growth.

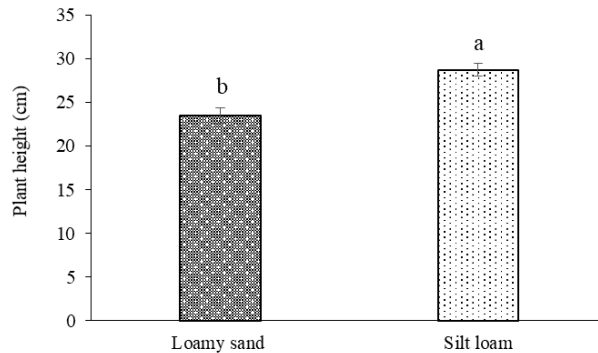


Figure 1. Effect of soil texture on plant height. Treatments with the same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$). Error bars show standard error of the mean ($n = 56$)

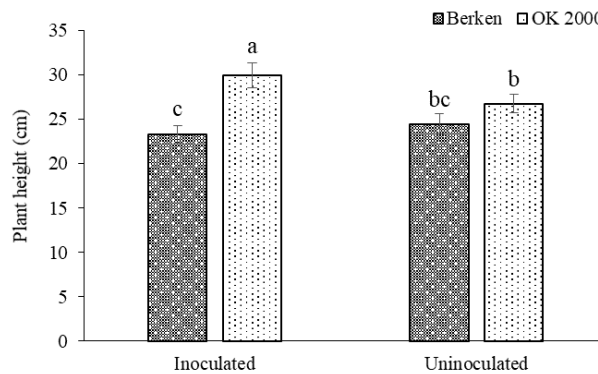


Figure 2. Effect of cultivar on plant height of mungbean grown in soil with and without *Bradyrhizobium* (group I) inoculation. Treatments with the same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$). Error bars show standard error of the mean ($n = 56$)

Number of pods per plant

Bradyrhizobium inoculation and soil texture significantly affected mungbean pod formation (Table 3). Over cultivars and soil textures, the maximum number of pods (12 pods/plant)

was observed for inoculated mungbean (a 15% increase) compared to uninoculated plants (Table 4). Anjum et al. (2006) found similar results when studying the impact of *Rhizobium* inoculation on mungbean and reported that inoculation with *Rhizobium* increased the number of pods per plant over the control. A pot experiment performed by Hussain et al. (2014) to determine the response of mungbean nodulation, growth and yield to *Rhizobium* inoculation revealed a 50% increase in the number of pods per plant compared to the control.

The comparison of the pod number per plant indicated that mungbean grown in silt loam soil had, on average, 12 pods/plant which represents a 21% increase compared to mungbean grown in loamy sand soil (Table 4). Similar results were obtained by Oke and Eyitayo (2010) when evaluating cowpea (*Vigna unguiculata* L. Walp.) growth under different fallow soils in Nigeria. They reported a significantly greater number of pods for plants grown in a sandy clay loam than a clay loam or sandy loam. Enhancement of pod formation under these soil textures could be attributed to greater concentration of organic matter, higher pH, greater percentage of exchangeable cations and greater water and fertilizer retention. The significant differences on number of pods per plant reported in our study can be attributed to the favorable conditions of silt loam soil for mungbean development. Some researchers, confirming our present results, also compared the number of pods per plant of legumes on different soil textures and reported a significant effect evidencing its importance on legume growth and yield (Ndema, Etame, Taffouo, & Bilong, 2010; Zhao, Jia, Y. Wang, M. Wang, & McGiffen Jr., 2015).

Table 4. Main effects of cultivar, inoculation, and soil texture on number of pods, seed weight, and plant residue weight per plant

| Treatments | | No. pods | Seed weight (g) | Plant residue (g) |
|--------------|--------------|-------------|-----------------|-------------------|
| Cultivar | Berken | 10.79±0.40a | 3.70±0.21b | 2.48±0.18b |
| | OK2000 | 10.93±0.40a | 4.25±0.18a | 3.18±0.18a |
| Inoculation | Inoculated | 11.75±0.50a | 4.28±0.20a | 2.99±0.12a |
| | Uninoculated | 9.96±0.44b | 3.68±0.20b | 2.67±0.12a |
| Soil texture | Loamy sand | 9.61±0.45b | 3.24±0.16b | 2.12±0.12b |
| | Silt loam | 12.11±0.43a | 4.72±0.13a | 3.54±0.15a |

Note. For each parameter, means within each treatment followed by different letter are significantly different [Fisher's protected LSD ($\alpha = 0.05$)].

Number of seeds per plant

The number of seeds per plant was significantly affected by mungbean inoculation (Table 3). Mungbean inoculation increased the number of seeds per plant of inoculated plants by 18% compared to uninoculated plants to an average of 84 seeds/plant (Figure 3). Hussain et al. (2014) evaluated the effects of *Rhizobium* inoculation on yield and yield attributes of mungbean. They found that inoculation significantly increased (23%) the number of seeds per pods compared to uninoculated plants. However, a study conducted by Kyei-Boahen, Savala, Chikoye, and Abaidoo (2017) on growth and yield responses of cowpea revealed that inoculation did not significantly increase number of seeds per pod compared to uninoculated plants on a Rhodic Ferralsol. The differences in number of seeds per plant of mungbean reported in our study could be attributed to genotypic differences (Peksen, Toker, Ceylan, Aziz, & Farooq, 2015; Razzaque et al., 2016).

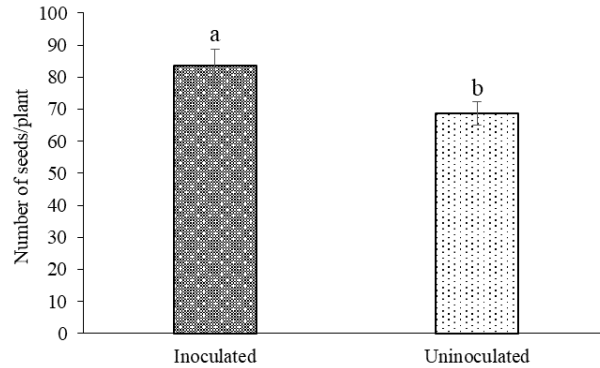


Figure 3. Effect of inoculation on number of seeds per plant of mungbean. Different letters on bars means significant difference between treatment according to Fisher's protected LSD ($\alpha = 0.05$). Error bars show standard error of the mean ($n = 56$)

The interaction effect of cultivar and soil texture on number of seeds per plant was statistically significant (Table 3). Figure 4 shows that the greatest number of seeds was recorded for Berken grown in silt loam soil with 95 seeds per pod while Berken grown in loamy sand produced the lowest number of seeds per plant (55 seeds/plant). Eugegrave, Jacques, Désiré, and Paul (2010) showed in their study that yield of cowpea significantly increased in plots with sandy clay loam with high sand content (72%), organic matter and exchangeable cations. In our study, the difference in number of seeds per plant can be explained by better performance of Berken in silt loam soil. Previous findings (Ahmad et al., 2015; Kumar & Sharma, 2009) have shown that higher seed yield of mungbean cultivars under different soil textures might be due to greater root system development and a subsequent increase in water and nutrient uptake.

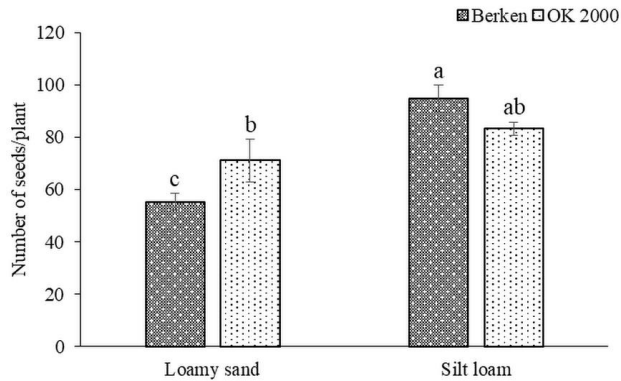


Figure 4. Effect of cultivar on number of seeds per plant of mungbean grown on a loamy sand and silt loam soils. For each soil texture, bars with the same letter are not significantly different according to Fisher’s protected LSD ($\alpha = 0.05$). Error bars show standard error of the mean ($n = 56$)

Seed weight

The seed weight of mungbean (g/plant) was significantly affected by cultivar, inoculation, and soil texture (Table 3). Among cultivars, the maximum seed weight of 4.25 g per plant was observed for OK2000 and represented a 13% increase compared to Berken (Table 4). M. Begum, M. Begum, Anwar, and Juraimi (2009) also studied the effect of cultivar on morphological characters, yield attributes and yield of mungbean varieties and reported BINA moog7 cultivar had the highest seed yield per plant compared to other varieties. Variations in yield among mungbean cultivars was explained by hereditary superiority, higher yield potential and greater translocation of nutrients (Ahmad et al., 2015; Sriphadet, Lambrides, & Srinives, 2007).

Inoculation of mungbean resulted in a 14% increase compared to uninoculated mungbean (3.68 g/plant) while growing in the silt loam soil resulted in a 31% increase in seed weight compared to the loamy sand (3.24 g/plant) (Table 4). Similar results were reported by Bhuiyan et al. (2008) who investigated the response of mungbean cultivars to *Bradyrhizobium* inoculation and reported a significant 27% increase in seed yield over control. The high yields of mungbean under

bacterial inoculation could be attributed to plant growth promoting substances in the rhizosphere and efficient nutrient uptake resulting in increased dry matter accumulation and greater photosynthate translocation to the seed (Zaidi, Khan, & Aamil, 2004). The field study conducted by Stajković-Srbinović, Kuzmanović, Mrvić, and Knežević-Vukčević (2011) to explore growth and yield components of mungbean under different soil types showed a 24% increase in bean yield in a clay loam soil compared to a heavy clay soil. Low seed yield of mungbean in heavy clay could be explained by reduced infiltration and slow drainage due to the prevalence of small pores in soil.

Bean plant residue weight

The means presented in Table 4 showed that OK2000 had the highest plant residue biomass (3.18 g) which was 22% greater than Berken. This result is in accordance with Bhuiyan et al. (2008) who reported that BARI Mung-2 mungbean cultivar produced significantly higher stover yield than two local mungbean cultivars. Mondal et al. (2012) demonstrated that higher production of plant residue in mungbean was explained by larger leaf area, greater branches per plant and increased plant height.

Residue mass of mungbean plants was also significantly affected by soil texture. Mungbean plants grown in the silt loam soil produced 40% more residue (3.54 g) than those plants grown in loamy sand soil. Similar to our results, Stajković-Srbinović et al. (2011) reported differences in the shoot dry yield of mungbean when grown under different soil conditions. They noted greater shoot dry matter of mungbean in clay loam texture compared to heavy clay soil. Cook et al. (1995) demonstrated from growth chamber and field experiments that high soil resistance characteristic of fined-textured soils could explain constraints to biomass production of mungbean. Zhao et al. (2015) reported that lower dry matter accumulation of peanut (*Arachis hypogaea* L.) on sandy soil compared to loam and clay soils was likely due to poor soil fertility in the sandy soil.

Protein content

Seed protein content was significantly different between cultivars (Table 3). Maximum protein content (25%) was obtained with the mungbean cultivar OK2000 (Figure 5). The critical review on the technological and nutritional potential of mungbean conducted by Dahiya et al. (2015) revealed variations in the reported protein concentrations ranging from 15-33% with a mean of 24%. Bhardwaj and Hamama (2016) observed no significant effects of cultivar on protein concentration of Berken and TexSprout seeds. Varietal differences in mungbean seed protein content are likely explained by differences in genetic constitution as reported by Hussain, Malik, Haji, and Malghani (2011), and Paul, Mozumder, Sayed, Akhtaruzzaman, and Akhtaruzzaman (2011).

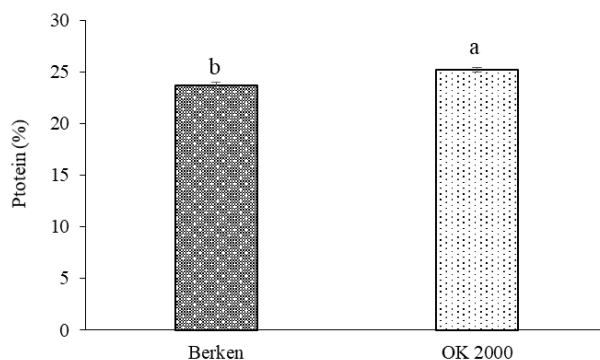


Figure 5. Effect of cultivar on protein content. Different letters on bars means significant difference between treatment according to Fisher's protected LSD ($\alpha = 0.05$). Error bars show standard error of the mean ($n = 56$)

Conclusion

Bradyrhizobium (group I) inoculation, cultivar, and soil texture affect yield and yield components of legume crops. There were significant differences in yield and yield attributes of mungbean due to the combined effects of variety (Berken and OK2000 cultivars), inoculum

treatment, and soil texture under greenhouse conditions indicated. Inoculation of OK2000 cultivar grown on silt loam soil seems a promising agronomic practice for improving yield of mungbean. *Bradyrhizobium* inoculation increased the number of pods and the number of seeds per plant, but did not increase seed weight. Plant height at maturity was greater when OK2000 was inoculated, but inoculation did not affect mature height of Berken mungbean indicating differential response by cultivar. Mungbean productivity was frequently influenced by soil texture with greater number of pods, seed weight and plant residue weight for plants grown in silt loam soil. These results indicating the significant effects of soil texture, inoculation, and cultivar on mungbean yield need to be confirmed by field experiments.

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Chapter 4 - Evaluation of pearl millet and mungbean intercropping systems in the semi-arid regions of Senegal

Abstract

Agricultural production in Senegal is constrained by rainfall variability, persistent droughts, and low soil fertility. These constraints have decreased pearl millet [*Pennisetum glaucum* (L.) R. Br.] yields in Senegal. Mungbean [*Vigna radiata* (L.) Wilczek], a short-duration and relatively drought-tolerant legume crop, is capable of improving soil fertility and performance of a companion crop. To investigate the potential of mungbean for increasing millet yields through intercropping, field experiments were conducted during the 2017 and 2018 growing seasons in Bambey (14°43'12" N, 16°36'41" W) and Nioro (13°45'0" N, 15°48'0" W) located within Senegal's west central (Peanut Basin) and Saloum agricultural regions, respectively. Intercropping treatments: sole millet (T₁), sole mungbean (T₂), and 23% (T₃), 43% (T₄), 47% (T₅), 62% (T₆), 125% (T₇) and 164% (T₈) of mungbean planting density in sole millet were laid out in a randomized complete block design and replicated four times. Measured parameters included yield parameters for millet panicle count and length, mungbean pod number, combined yields, land equivalent ratio (LER), canopy cover, and normalized difference vegetation index (NDVI). Combined millet and mungbean seed yields were 35% to 100% higher ($P \leq 0.05$) under intercropping systems compared to sole millet at Bambey and Nioro, respectively. Similarly, LER was always greater than unity (>1) for the various intercropping densities compared to monoculture. Mean canopy cover estimates and NDVI values increased by 60% and 30% in millet-mungbean intercropping over millet grown alone, respectively. These combined yield increases were obtained without fertilizer applications. They suggest that

optimizing mungbean density in pearl-millet based systems can increase the combined yield in a low-input and/or high-risk environment in Senegal.

Keywords: Mungbean, Millet, Yields, Intercropping, Senegal

Core Ideas

- Intercropping millet with mungbean significantly increased combined seeds yield by 35% in the Senegalese West Central Agricultural Region and by 100% in the Saloum Agricultural Region compared to millet grown alone.
- Millet-mungbean intercropping significantly increased LER (always >1), canopy cover (up to 60%), and NDVI values (up to 30%) over sole millet and tended to increase with increasing mungbean density.
- Optimizing mungbean density in millet systems would improve the productivity of pearl millet-based systems in semi-arid regions of Senegal.

Introduction

In Senegal, agricultural production systems are of paramount importance to the national economy and the livelihoods of rural populations (Mertz et al., 2009; Diatta et al., 2016b; Tankari and Traore, 2017). However, these agricultural systems, like those of other sub-Saharan African countries, face various constraints which are mainly due to climate variability and increased frequency of extreme weather events, intrusion of salt water, and pest and disease incidence (Mbow et al., 2008; Kotir, 2011; Roudier et al., 2011; Diatta, 2016; Faye et al., 2019; Daryanto et al., 2020). In addition, poor agricultural practices which result in land degradation and stagnation or even a decline in agricultural production at a time when population growth is accelerating at 2.7% per year (The World Bank, 2018). Senegalese agriculture is still extensive

and essentially rain-fed (Diangar et al., 2004; Fall and Lo, 2009; Gaudreau and Gibson, 2015). It is also characterized by small family farms whose access to inputs (e.g. fertilizers, pesticides, equipment) is limited by the low income of farmers (Barrett and Bevis, 2015), limited access to credit (Seck, 2017) but also the limited use of soil fertility improvement and management practices (Settle and Garba, 2011; Diatta et al., 2016a; Tounkara et al., 2020).

Several studies propose sustainable solutions to the deterioration of cropland fertility and the decline in yields of major crops such as pearl millet, peanut (*Arachis hypogaea* L.), maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], cowpea [*Vigna unguiculata* (L.) Walp.], and rice (*Oryza sativa* L.) in Senegal. Research has shown that the adoption of adequate soil restoration and management practices and the implementation of climate change adaptation strategies can slow and even reverse the degradation of agricultural production systems (Roudier et al., 2011; Connolly-Boutin and Smit, 2016).

Agricultural production is mainly intended to meet local needs (food crops and animal production) in Senegal. Millet, a dominant staple crop among cereals, covers more than 63.5% of the land under cereal production with an annual production estimated at 574,000 tonnes in 2018 for a yield of 0.7 t ha⁻¹ (FAOSTAT, 2020). Unlike sorghum and maize, which are confined to more humid areas, millet is found throughout the country on all soil types and in all agro-ecological zones because of its broad adaptation. It is also usually grown under rainfed and extensive conditions with few or no inputs, resulting in on-farm yields ranging from 0.5 t to 0.6 t ha⁻¹ in farmers' fields (Kouakou et al., 2015). Low yields of major crops such as millet have led to Senegal's dependence on food imports (Van den Broeck et al., 2018). To offset the decline in millet productivity, it is therefore necessary to develop viable and accessible alternatives for farmers and innovative practices for good agricultural production (Trail et al., 2016).

Intercropping of cereals and legumes can restore and improve soil fertility, diversify millet production systems, and provide fodder for animal feed (Matusso et al., 2014; Diatta et al., 2019a; Daryanto et al., 2020). Intercropping, which means planting two or more crops in the same field simultaneously (Gomez and Gomez, 1983), is a very effective approach in the use of resources and the promotion of soil protection and the gradual restoration of natural soil fertility (Brooker et al., 2015). In addition, intercropping systems are well-known and very common, old practices to farmers in the tropics (Francis et al., 1976). Several studies have demonstrated the importance of legumes in improving biological, organic and even mineral soil quality (Gebu, 2015; Maman et al., 2017; Franke et al., 2018; Vanlauwe et al., 2019). Other authors have found that the combination of legumes with cereal crops promotes improved agronomic performance and yields of cereal crops (Trail et al., 2016; Diatta et al., 2019a). Despite the use of multiple crop combinations such as millet-peanut, millet-cowpea, and millet-sorghum-cowpea in Senegal, there is still a recurrent decline in millet yields (Diangar et al., 2004; Mbaye et al., 2014). This lack in yield improvement could be explained by the use of a limited number of legumes and lack of appropriate recommendations for species sowing densities and spatial arrangement.

Thus, the introduction of mungbean into Senegalese agriculture could help increase agricultural production through farming system diversification (Abaye et al., 2015; Diatta et al., 2018). Mungbean is an annual short-cycle (60-65 days) legume of the *Fabaceae* family and is widely grown in the tropical and subtropical regions of the world (Pataczek et al., 2018; Nair et al., 2019). It is mainly grown in rotation with cereals and, in symbiosis with *Rhizobium* ssp. fix up to 110 kg N/ha, which could help meet its own nitrogen needs and those of the associated or next crop (Shah et al., 2003; Shahida and Khan, 2016). Mungbean also represents an opportunity to produce protein-rich foods for smallholders under changing climatic conditions (Keatinge et

al., 2011; Arsenault et al., 2015; Foyer et al., 2016). The only reported study in Senegal of millet-mungbean intercropping (1:1 row ratio) demonstrated yield improvement, particularly for the millet (Trail et al., 2016). However, the appropriate planting arrangement and density of mungbean in intercropped millet systems has not been studied in Senegal.

Our study evaluated the effects of intercropping mungbean on pearl millet yield and yield attributes in the semi-arid region of Senegal. Field experiments were conducted during the 2017 and 2018 growing seasons at the Senegalese Institute of Agricultural Research (ISRA) stations of Bambey and Nioro. Specifically, the objectives of the field studies were to: i) investigate the effects of intercropping millet with multiple planting densities of mungbean on millet and mungbean yield components; ii) evaluate millet-mungbean intercropping density and spacing on yields, combined yields, and LER; and iii) compare canopy ground cover and NDVI values of intercropped millet and grown alone.

Materials and methods

Site description

The field experiments were conducted in Bambey (14°43'12" N, 16°36'41" W; Diourbel region) and in Nioro du Rip (13°45'0" N, 15°48'0" W; Kaolack region). The climate in Diourbel is Sahelo-Sudanian and Coastal Sudanian and characterized by a mean annual temperature of 27 °C to 28 °C and mean annual rainfall values of about 520 mm from 1991 to 2016 (Climate Research Unit of East Anglia, 2019), most of which occurs during a three-month rainy season (Mbow et al., 2008; Mertz et al., 2009). The Kaolack region is characterized by a Coastal Sudanian climate with an average annual rainfall of 900 mm from 1991 to 2016 (Climate Research Unit of East Anglia, 2019). Table 1 summarizes monthly rainfall (mm), temperature (°C), and relative humidity (%) in Bambey 2017, Bambey 2018, and Nioro 2018 between June

and November. The annual rainfall values were 527 mm in 2017 and 448 mm in 2018 for Bambey corresponding to 34 and 27 days of more than 1 mm of rainfall, respectively.

Situated in the West Central Agricultural Region or “Peanut Basin” (25,915 km²), the soil types in Diourbel are iron-rich tropical sandy soils, and slightly leached (Tappan et al., 2004). The predominant soils in Kaolack located within the “Saloum Agricultural Region” (6413 km²) are iron-rich tropical and ferralitic soils; loamy sands over fine sandy loam at depth (Tappan et al., 2004). Table 2 presents the chemical properties of the soil at each experimental site.

Table 1: Monthly rainfall (mm), temperature (°C), relative humidity (%) and number of days receiving more than 1 mm of rainfall (DoR) between June and November for Bambey 2017, Bambey 2018, and Nioro 2018.

| Sites | Parameters | June | July | Aug. | Sep. | Oct. | Nov. | Total |
|-------------|-----------------------|------|------|------|------|------|------|-------|
| Bambey 2017 | Rainfall (mm) | 125 | 120 | 177 | 88 | 18 | 0 | 528 |
| | Temperature (°C) | 31 | 30 | 28 | 29 | 31 | 29 | |
| | Relative humidity (%) | 71 | 82 | 84 | 79 | 71 | 65 | |
| | DoR | 3 | 9 | 13 | 6 | 3 | 0 | 34 |
| Bambey 2018 | Rainfall (mm) | 33 | 14 | 161 | 205 | 33 | 2 | 448 |
| | Temperature (°C) | 30 | 30 | 30 | 29 | 30 | 28 | |
| | Relative humidity (%) | 65 | 70 | 74 | 82 | 75 | 55 | |
| | DoR | 1 | 2 | 9 | 11 | 3 | 1 | 27 |
| Nioro 2018 | Rainfall (mm) | 16 | 99 | 205 | 314 | 49 | 0 | 683 |
| | Temperature (°C) | na† | na | na | na | na | na | |
| | Relative humidity (%) | na | na | na | na | na | na | |
| | DoR | 1 | 6 | 13 | 12 | 5 | 0 | 37 |

† na, not available.

Table 2. Summary of soil chemical properties at Bambey 2017, Bambey 2018, and Nioro 2018 sites.

| Sites | pH [†] | Total N —— % ‡ —— | Total C | C/N | NO ₃ ⁻ — mg kg ⁻¹ § — | NH ₄ ⁺ | P | K | Ca | Mg |
|-------------|-----------------|----------------------|---------|------|---|------------------------------|----------------------------------|-----|-------|------|
| | | | | | | | ———— mg kg ⁻¹ ¶ ————— | | | |
| Bambey 2017 | 8.0 | 0.03 | 0.28 | 9.9 | 0.1 | 0.5 | 15.6 | 5.2 | 209.1 | 43.6 |
| Bambey 2018 | 6.7 | 0.02 | 0.23 | 11.2 | 0.1 | 0.2 | 4.4 | 5.5 | 69.7 | 14.9 |
| Nioro 2018 | 5.6 | 0.03 | 0.38 | 12.4 | 0.1 | 0.3 | 1.0 | 4.2 | 27.5 | 4.7 |

† pH: soil (1:1 soil: deionized water mixture on a volumetric basis).

‡ Total carbon (C) and total nitrogen (N): vario MAX CNS Element Analyzer.

§ Inorganic NO₃⁻ and NH₄⁺: 2M KCl extraction.

¶ Plant available nutrients: soil (Mehlich 1 solution).

Experimental design

The experiment was laid out as a randomized complete block design composed of eight treatments replicated four times. The first treatment was millet only (control treatment, T₁) whose density and spacing would represent the conventional practice followed by farmers in Senegal (Gupta, 1984; Fall and Lo, 2009). Millet spaced 0.9 by 0.9 m is commonly practiced by smallholders to allow animal traction used to loosen the soil for a good circulation of water and air in depth, promote root development, infiltration of rains on the surface, and weed control, and to mix organic inputs and soil amendments (Nicou, 1977). Mungbean grown alone and intercropped with millet represented the following treatments summarized in Figure 1. The proportions of mungbean grown with millet corresponded to their relative density compared to mungbean density in pure cultivation (T₂). Experimental plots measured 4.5 by 2.7 m and were

separated by 1.8 m buffer. Millet seeds were sown in hills with 0.9 by 0.9 m spacing and thinned to three plants per hill for a density of 12,346 hills per hectare. Three mungbean seeds were sown per hill at 0.5 by 0.5 m and thinned to one plant for a plant density of 40,000 hills per hectare when grown alone. Planting densities and arrangements for millet and mungbean grown alone and intercropped are illustrated in Figure 1.

Experimental plots were planted manually under rainfed conditions with no fertilizer additions. Millet and mungbean were planted on 19 July 2017 in Bambey. In 2018, all crops were planted on 27 and 30 of July in Nioro and Bambey, respectively. Prior to planting, mungbean seeds were mixed with arabic gum and inoculated with *Bradyrhizobium* spp. (group I) at a rate of 10 g inoculant kg⁻¹ seed to allow thorough coating. All experimental sites had been under millet cultivation prior to initiation of these studies. During early vegetative stages, weed management system included manual weeding. Seeds of millet (Souna III) were obtained from National Center for Agronomic Research (CNRA) laboratory. Mungbean seed (Berken) was purchased from Oklahoma Foundation Seed Stocks (Stillwater, Oklahoma, USA).

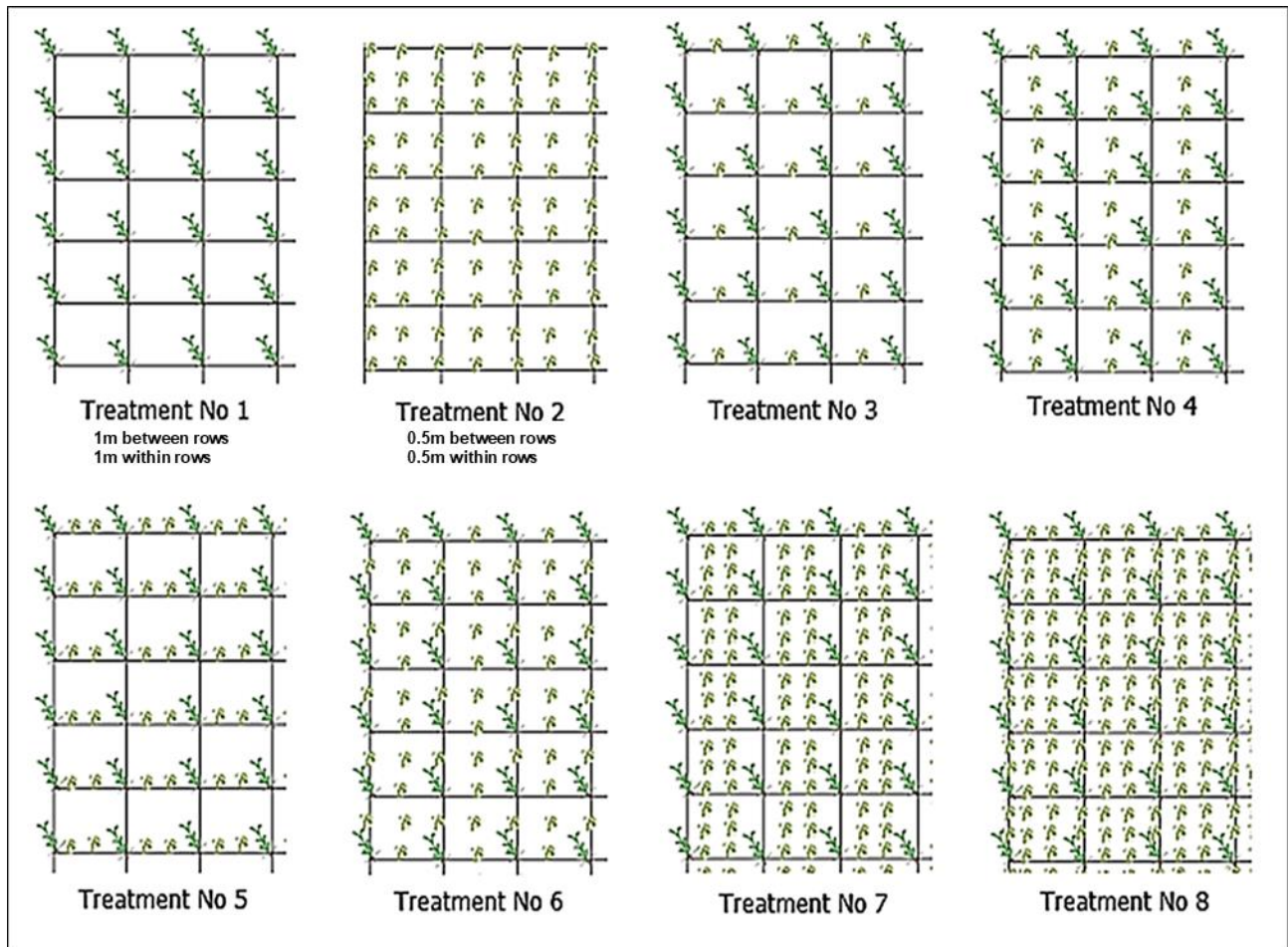


Figure 1. Planting densities and arrangements for millet and mungbean grown alone and intercropped. Millet alone (T_1), mungbean alone (100%, T_2), 23% mungbean (T_3), 43% mungbean (T_4), 47% mungbean (T_5), 62% mungbean (T_6), 125% mungbean (T_7) and 164% mungbean (T_8) grown in combination with the millet.

Data collection

Millet biomass and grains were hand-harvested from the center (7.6 m^2) of each plot at maturity and oven dried for 2-3 days at 60°C to constant weight and yields reported based on 12.5% moisture content. Additional millet parameters included plant height (cm), stem diameter (cm), number of panicles, and panicle length (cm). To determine the effects of mungbean intercropping on millet components, each millet plant was separated into

inflorescence, leaf, and stem. Dry weight was measured for each parameter. Mungbean pods were hand harvested from the central rows (7.6 m²) of each experimental plot. Mungbean yield was measured at 12.5% moisture content. Average plant height (cm) and number of pods were recorded at harvest.

To evaluate the advantage of intercropping millet with mungbean over millet grown alone, the total land equivalent ratio (LER) was calculated. LER is defined as a measure of crop productivity and resource-use efficiency of intercropped systems compared to sole cropping (Mead and Willey, 1980). LER greater than unity indicates greater land utilization efficiency and yield per unit area of intercropping over sole cropping, and is calculated as follow:

$$LER = LER_{Mung} + LER_{Mil} = (Y_{IMung} / Y_{SMung}) + (Y_{IMil} / Y_{SMil})$$

where: Y_{IMung} and Y_{IMil} are mass yields per unit area of intercropped mungbean seeds and millet grains respectively and Y_{SMung} and Y_{SMil} are mass yield per unit area of sole cropped mungbean seeds and millet grains respectively.

Canopy cover measurements were taken at sowing, during vegetative growth, at flowering, during pod development, and at maturity with the Canopeo app (Shepherd et al., 2018). Canopeo, an image analysis application, uses color values based on the red-green-blue (RGB) system and fractional green canopy cover which ranges from 0 to 1 corresponding to no or 100% green canopy cover, respectively (Patrignani and Ochsner, 2015). Normalized difference vegetation index (NDVI) was also collected during the growing season with a Trimble Greenseeker hand-held optical sensor (Trimble Navigation, Sunnyvale, CA). To help determine N differences between millet grown alone and intercropped with mungbean, readings were taken on millet plants from the harvested area.

Statistical analysis

Millet and mungbean yields, canopy cover, and NDVI data were analyzed using SAS JMP Pro version 15.0.0 statistical software (SAS Institute Inc., Carey, NC). Prior to the analysis of variance (ANOVA), normal distribution of data was assessed using the Anderson-Darling goodness of fit test (Laio, 2004) and homogeneity of variance evaluated using Levene test (Gastwirth et al., 2009). When assumptions of normal distribution of data and homoscedasticity were not met, data were transformed using Box-Cox power transformation (Box and Cox, 1964; Osborne, 2010). ANOVA was then used to test the effects of the millet and mungbean intercropping on the studied parameters. Treatment means were separated using Fisher's protected LSD test at $\alpha = 0.05$ level of probability when values were significant. No data were collected at Nioro site in 2017 due to flooding. Due to interactions between sites and intercropping treatments, data are presented individually by site.

Results

Millet, mungbean, and combined intercropping yields

Pearl millet and mungbean yields under intercropping and monocropping systems are summarized in Table 3. Millet grain yields were not affected by intercropping treatments at either site, but there was a significant yield difference ($P < 0.05$) between sites (Table 3). Millet produced the highest grain yield of 1023 kg ha⁻¹ at Nioro in 2018 which was 52% and 116% greater than millet yield at Bambey, 2017 or Bambey, 2018, respectively (Table 4).

There was a significant interaction between intercropping treatment and site for mungbean seed yield (Table 3), though seed yield tended to increase with increasing mungbean density, as expected. The mean mungbean seed yields in millet intercropped with 164% (T₈) and

125% (T₇) of mungbean were significantly ($P < 0.05$) greater (44%) than that in sole mungbean (T₂) in Bambey, 2017 (Fig. 2). At Bambey and Nioro in 2018, mungbean grown alone always produced the highest yields of 444 and 544 kg ha⁻¹ compared to other intercropping treatments, respectively (Fig. 2).

Table 3. Analysis of variance of site, intercropping treatments, and interaction of main effects for yield and yield components of millet and mungbean, canopy cover, NDVI, LER.

| Source | df | Millet | | | | | Mungbean | | | Combined | |
|------------------|----|---------------------|---------------|--------------|--------------|------|---------------------|--------------|-------------|---------------------|-----|
| | | Yield | Panicle count | Plant height | Canopy cover | NDVI | Yield | Plant height | Number pods | Yield | LER |
| | | kg ha ⁻¹ | per plot | cm | % | | kg ha ⁻¹ | cm | per plot | kg ha ⁻¹ | |
| Site | 2 | *** | *** | *** | *** | *** | *** | *** | *** | *** | ns |
| Treatment | 7 | ns† | ns | ns | *** | *** | *** | *** | *** | *** | *** |
| Site × Treatment | 14 | ns | * | ns | ns | ns | * | *** | ** | * | ns |
| Model | 23 | | | | | | | | | | |
| Error | 72 | | | | | | | | | | |
| Total | 95 | | | | | | | | | | |

* Significant at $\alpha = 0.05$.

** Significant at $\alpha = 0.01$.

*** Significant at $\alpha = 0.001$.

† ns– nonsignificant at $\alpha = 0.05$.

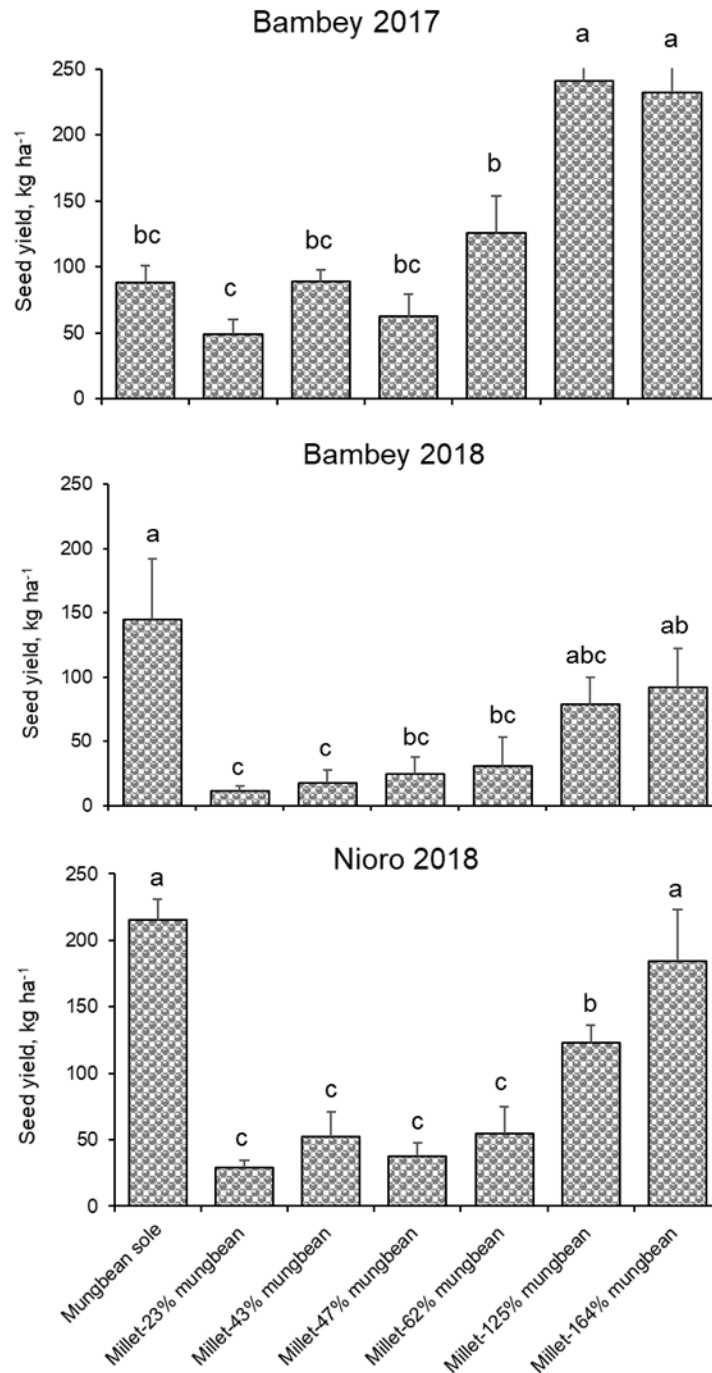


Figure 2. Mungbean yields in monocropping and intercropping at Bambeby 2017, Bambeby 2018, and Niro 2018. Treatments connected by dissimilar letters are significantly different at $\alpha = 0.05$ according to Fisher's protected LSD and error bars represent standard error of the mean ($n = 84$).

Combined seed yields of millet and mungbean were significantly ($P < 0.05$) affected by an intercropping by site interaction (Table 3). At Bambey 2017, combined seed yields were significantly ($P < 0.05$) higher than those of monocropped systems. Maximum seed yields of 914 and 905 kg ha⁻¹ were recorded for millet intercropped with 47% (T₅) and 62% (T₆), respectively, which corresponded to a 38 and 36% increase compared to millet grown alone (Fig. 3). Similarly, significant differences ($P < 0.05$) were observed for combined yields at Bambey 2018, where millet + 62% mungbean (T₆) intercropping treatment yielded 86% more than monocropped millet (452 kg ha⁻¹) (Fig. 3). At Nioro 2018, intercropping systems significantly ($P < 0.05$) produced more combined seed than millet grown alone (Fig. 3). A maximum combined seed yields of 1468 kg ha⁻¹ in millet intercropped with 125% mungbean (T₇) was recorded, an 85% increase compared to millet alone (Fig. 3).

Intercropping mungbean with millet did not significantly ($P > 0.05$) influence the inflorescence, leaf, and stem dry biomass of millet plants despite its presence at high densities (up to 164% compared to T₂). Conversely, differences ($P < 0.05$) were observed for millet parameters among sites (Table 4). The highest recorded values for plant height and panicle length were in Nioro 2018 while greater stem diameter and dry biomass were recorded at Bambey 2017 (Table 4). At Bambey in 2018, millet grown alone produced more panicles than other intercropping treatments (Table 5). However, number of panicles was not significant ($P > 0.05$) between treatments at Bambey 2017 and Nioro 2018. At all sites, the number of panicles did not always significantly differ between treatments, but millet grain yields were significantly different. Statistical differences were found for millet panicle length between sites, with Nioro and Bambey 2018 recording the highest panicle length, 49 and 48 cm respectively, compared to Bambey 2017 (37 cm) (Table 4).

For mungbean, when the number of pods was significantly different between treatments, seed yield also differed between mungbean grown alone and intercropped. The greatest number of pods for Bambey 2017 was 125% (T₇) and 164% (T₈) of mungbean. Sole mungbean (T₂) and millet-125% mungbean (T₇) produced more pods at Bambey and Niroo in 2018 (Table 5). At Bambey in 2017, intercropped mungbean with millet had higher plant height than when mungbean was grown alone. Mungbean plants under 125% (T₇) and 164% (T₈) density recorded the highest plant heights of 149 and 144 cm, respectively, which corresponded to over 160% increase compared to mungbean grown alone (Table 5). Conversely in 2018, there was no significant difference for mungbean plant height between the two sites (Table 5).

Table 4. Millet panicle length, plant height, stem diameter, and dry biomass at Bambey 2017, Bambey 2018 and Niroo 2018.

| Site / Parameters | Panicle length | Plant height | Stem diameter | Grain yield | Stem biomass | Leaf biomass | Panicle biomass | Dry biomass |
|-------------------|----------------|--------------|---------------|---------------------|--------------|--------------|-----------------|-------------|
| | cm | | | kg ha ⁻¹ | | | | |
| Bambey 2017 | 37b* | 175b | 2a | 671c | 4176a | 4924a | 8317a | 17416a |
| Bambey 2018 | 48a | 137c | 1b | 473b | 1369c | 305c | 483c | 2157c |
| Niroo 2018 | 49a | 216a | 2a | 1023a | 2534b | 1144b | 1926b | 5604b |

* Means within each column followed by dissimilar letters are significantly different according to Fisher's protected LSD ($\alpha = 0.05$).

Table 5. Number of millet panicles and mungbean pods by treatment at Bambey 2017, Bambey 2018 and Nioro 2018.

| Site / Treatment | T ₁ † | T ₂ ‡ | T ₃ § | T ₄ ¶ | T ₅ †† | T ₆ ‡‡ | T ₇ §§ | T ₈ ¶¶ |
|---|------------------|------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|
| <u>Number of panicles/7.6 m²</u> | | | | | | | | |
| Bambey 2017 | 62a* | -- | 62a | 57a | 64a | 60a | 58a | 60a |
| Bambey 2018 | 59a | -- | 38c | 39bc | 44bc | 53ab | 38c | 31c |
| Nioro 2018 | 44a | -- | 49a | 53a | 45a | 49a | 53a | 48a |
| <u>Number of pods per plot</u> | | | | | | | | |
| Bambey 2017 | -- | 379bcd | 287e | 356cde | 311de | 404bc | 509a | 476ab |
| Bambey 2018 | -- | 444a | 158c | 196c | 205c | 219bc | 333ab | 370a |
| Nioro 2018 | -- | 544a | 278c | 321c | 301c | 347c | 432b | 530a |
| <u>Mungbean plant height, cm</u> | | | | | | | | |
| Bambey 2017 | -- | 55bc | 30c | 55bc | 39bc | 78b | 149a | 144a |
| Bambey 2018 | -- | 37a | 36a | 38a | 36a | 39a | 40a | 35a |
| Nioro 2018 | -- | 45a | 40a | 41a | 41a | 42a | 43a | 47a |

† T₁: Millet sole; ‡ T₂: Mungbean sole; § T₃: Millet-23% mungbean; ¶ T₄: Millet-43% mungbean; †† T₅: Millet-47% mungbean; ‡‡ T₆: Millet-62% mungbean; §§ T₇: Millet-125% mungbean; ¶¶ T₈: Millet-164% mungbean

* Means within each row followed by dissimilar letters are significantly different according to Fisher's protected LSD ($\alpha = 0.05$).

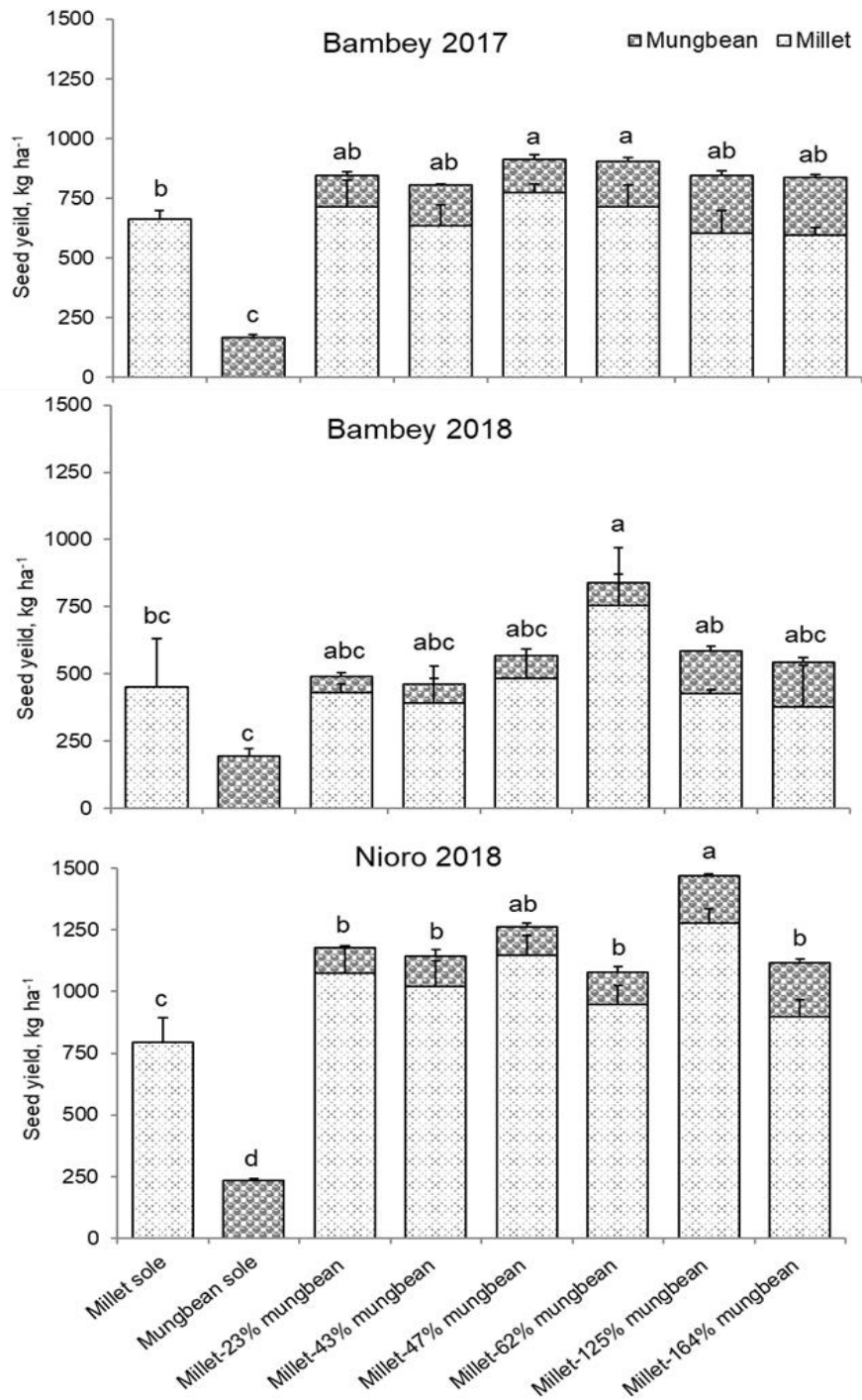


Figure 3. Millet and mungbean combined seed yield between treatments at Bambey 2017, Bambey 2018 and Niro 2018. Treatments connected by dissimilar letters are significantly different at $\alpha = 0.05$ according to Fisher's protected LSD and error bars represent standard error of the mean ($n = 96$).

Land equivalency ratios

Land equivalent ratio (LER) indicates the advantage of intercropping over a monocropping system (Oseni, 2010). In the present study, data for partial LER indicated yield advantage for all intercropped millet compared to monocropped millet, except for millet with 164% mungbean (Table 6). Partial LER of mungbean ranged from 0.51 to 1.09 for intercropped mungbean (Table 6). However, only partial LER of millet-125% mungbean (T₇) (1.04) and 164% of mungbean (T₈) (1.09) were greater than unity, indicating more seeds were produced per unit of land compared to mungbean grown alone.

Total LERs for the different intercropping systems were all greater than unity, demonstrating that intercropping was more efficient in land use than monocropping systems for seed yields. In addition, LER values showed an increasing trend with increasing mungbean density. The maximum LERs were 2.11 and 2.31 under millet-62% (T₆) and 125% mungbean (T₇) plant density, respectively, indicating that increasing mungbean density at 62% and 125% of standard mungbean plant density in millet increased land use productivity (Table 6).

Table 6. Land equivalency ratios for millet and mungbean in monocropping and intercropping systems.

| Intercropping treatments | Land Equivalency Ratio | | |
|--------------------------|------------------------|----------|--------|
| | Millet | Mungbean | Total* |
| Millet sole | 1.00 | -- | 1.00b |
| Mungbean sole | -- | 1.00 | 1.00b |
| Millet-23% mungbean | 1.25 | 0.51 | 1.76a |
| Millet-43% mungbean | 1.10 | 0.63 | 1.74a |
| Millet-47% mungbean | 1.39 | 0.60 | 1.99a |
| Millet-62% mungbean | 1.39 | 0.72 | 2.11a |
| Millet-125% mungbean | 1.26 | 1.04 | 2.31a |
| Millet-164% mungbean | 0.94 | 1.09 | 2.03a |

* Means within each column followed by dissimilar letters not significantly different at $\alpha = 0.05$ according to Fisher's protected LSD.

Canopy cover

Percent canopy cover values differed among intercropping treatments at different stages of crop development, but followed the same pattern during the growing season (Table 3).

Canopy cover increased with increasing mungbean density, and values were related to increasing density of mungbean ($r = 0.68$). The percent canopy cover of the monocropping systems was significantly lower ($P < 0.05$) than that of intercropping systems due to low shade cover from pearl millet. Millet grown alone generally recorded the lowest percent cover estimates, while intercropped millet with mungbean recorded, on average, 112% higher canopy cover over sole millet. The maximum canopy cover estimates for all treatments were recorded at flowering with T₈ reaching 63%, while millet grown alone (T₁) recorded 30% cover (Fig. 4). Maximum density of mungbean (T₈; 164%) in millet had the highest canopy cover estimates over the growing season and corresponded to a significant increase over millet alone of 237% at vegetative stage, 110% at flowering, 167% at pod development, and 182% at maturity stages (Fig. 4).

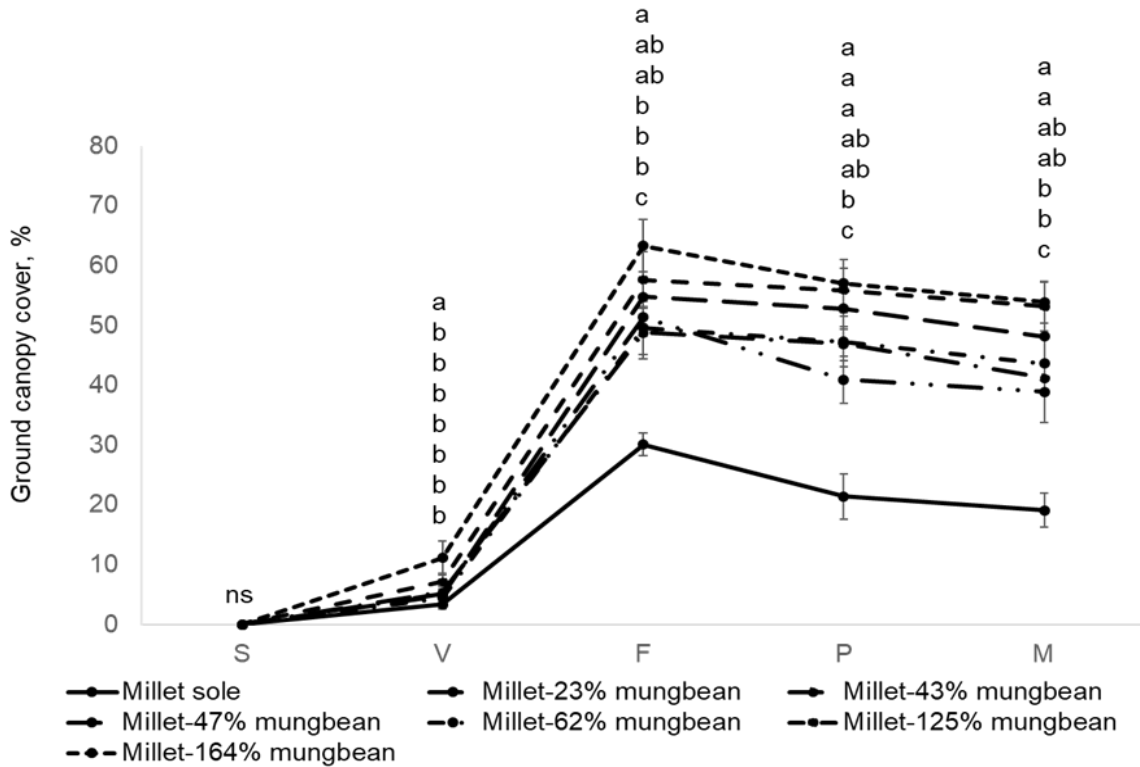


Figure 4. Percentage canopy cover of millet monocrop, mungbean monocrop, and millet + mungbean intercropping (S= Sowing, V= Vegetative, F= Flowering, P= pod development, M= Maturity). Treatments with dissimilar letters are significantly different at $\alpha = 0.05$ according to Fisher's protected LSD and error bars represent standard error of the mean ($n = 96$).

Normalized Difference Vegetation Index (NDVI)

NDVI is a standardized means of estimating plant health status (Gamon et al., 1995). Positive NDVI values correspond to green vegetation while negative values indicate little or no plant cover. NDVI values under intercropping treatments were significantly higher ($P < 0.05$) than that of sole millet at the same plant development stage. On average, a 16% increase was noted for intercropped millet with mungbean compared to millet monocrop (Fig. 5). However, NDVI values for sole millet and millet-mungbean intercropping reflected similar patterns from sowing to pod development stage. After the pod development stage, NDVI of millet intercropped

with 47 and 62% of mungbean increased while values of the remaining treatments decreased at maturity. Significantly lower NDVI values were reported throughout the growing season for millet grown alone compared to intercropped millet. At the vegetative stage, pod development, and maturity, NDVI values of millet intercropped with the highest density of mungbean (T₈; 164%) had the highest NDVI values, which significantly increased 51%, 21%, and 27% over millet grown alone, respectively (Fig. 5).

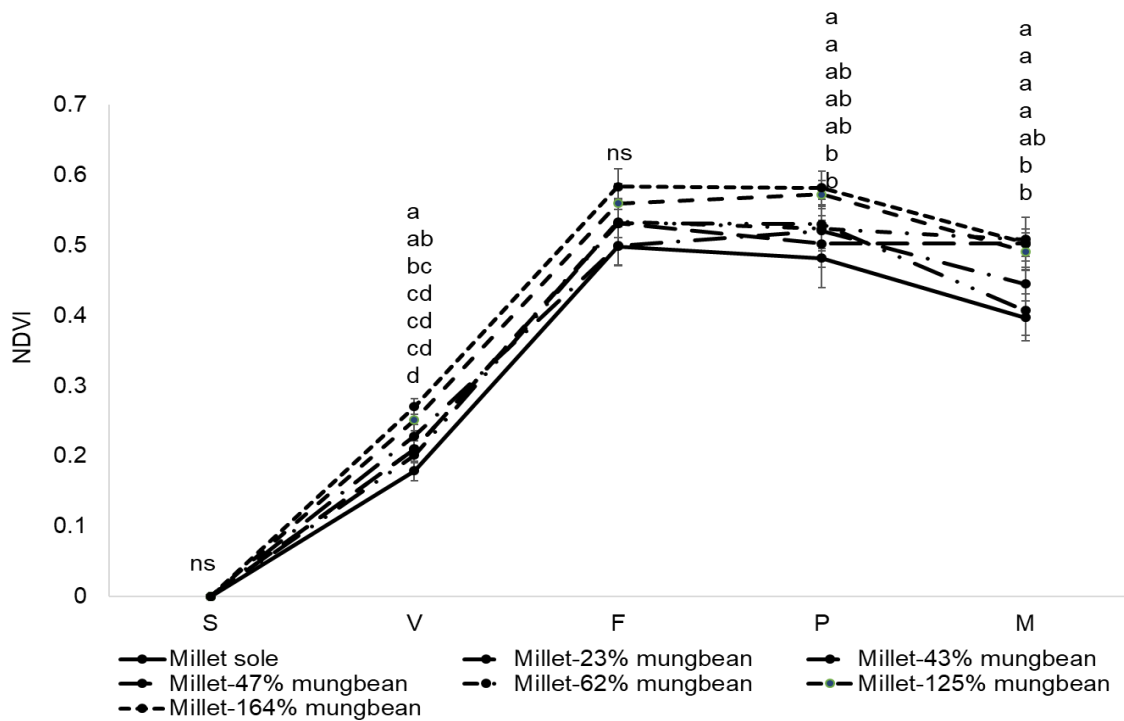


Figure 5. Normalized difference vegetation index (NDVI) of millet monocrop, mungbean monocrop, and millet + mungbean intercropping (S= Sowing, V= Vegetative, F= Flowering, P= pod development, M= Maturity). Treatments with dissimilar letters are significantly different at $\alpha = 0.05$ according to Fisher's protected LSD and error bars represent standard error of the mean ($n = 96$).

Discussion

Intercropping millet with mungbean did not significantly increase millet yields. Yield varied by sites with Nioro 2018 recording the highest grain yields. Despite being intercropped at high mungbean density (up to 164%), millet yields were not significantly reduced, demonstrating that mungbean did not adversely affect millet grain yield at those sites. Trail et al. (2016) also studied the effect of 1:1 millet-mungbean intercropping in Senegal and reported a grain yield increased by 36% for intercropped millet with mungbean compared to monocropped millet. Similar results were reported by Reddy et al. (1992) and Bationo et al. (1998) who reported a 15% increase in Niger and 30% increase in West Africa for intercropped millet compared to millet grown alone. In contrast, others studies have revealed reduction in millet yields under millet-legume intercropping systems. Ntare (1990) reported yield decrease varying from 11 to 75% in Niger while Diangar et al. (2004) found up to 24% decrease in millet yields in Senegal. A recent study conducted by Maman et al. (2017) revealed that pearl millet intercropped with cowpea in Mali yielded 18% less than millet monocropping.

Intercropped mungbean generally had lower yield than mungbean grown alone except for mungbean grown at high density (over 125% plant density). Similar to our results, Trail et al. (2016) and Reddy et al. (1992) both reported decreases in intercropped legumes compared to monocropped legumes by 50% et 48%, respectively. Reduction of cowpea seed yield by 47% was reported by Mohammed et al. (2008) due to intercropping with millet. Ntare (1990) intercropped millet and cowpea and found significant reduction in cowpea yield when grown in association with millet. However, monocropped and intercropped legumes were grown at lower densities in these field trials than the present study. The differences in mungbean yield in the present study is explained by increased planting density in intercropping treatments. The T₈,

which corresponds to 164% mungbean in millet sole, despite having the highest plant density, did not always result in the highest mungbean yield. The seeding rate of mungbean in intercropping could thus be reduced relative to its density in monoculture without significantly reducing yield (Matusso et al., 2014).

Combined seed yields of millet and mungbean were consistently higher in association than in monoculture. Nevertheless, we observed relatively low total yield in this study compared to yield reported in other intercropping experiments. The increase for total seed yield under intercropping in the present study is similar with Trail et al. (2016) when he intercropped millet with mungbean in Senegal and got a total seed yield of 2000 kg ha⁻¹. Naresh et al. (2017) reported greater yield of millet and mungbean under intercropping compared to monoculture and attributed it to beneficial interactions between millet and legumes. Ghilotia et al. (2015), by contrast, reported a decrease in combined grain yield compared to millet grown alone due to competition of associated crops for soil nutrients and environmental resources. In the present study, poor soil fertility associated with poor plant stand, insect pests, and low rainfall and early cessation might have resulted in relatively lower combined seed yields. Among intercropping treatments in Bambey, millet grown with 62% mungbean density (T₆) produced the highest combined seed yields both in 2017 and 2018, while millet-125% mungbean (T₈) yielded more at Nioro in 2018. The percentage yield increases were 60% in Bambey and 85% at Nioro 2018. Optimum mungbean density increased combined seed yields and is attributed to increased mungbean yield without decreasing millet yield. Differences in yields between sites could be due to variations in amounts of rainfall (488 mm at Bambey sites on average and 683 at Nioro 2018), indicating the need to consider site specificity for selecting the appropriate intercropping systems. Diangar et al. (2004) also reported higher pearl millet and cowpea yields in Central-

south region compared to Central-north region in Senegal due lower rainfall in the north. Singh et al. (2011) and Begum et al. (2009) investigated the effects of mungbean plant density and concluded that optimum row and plant spacing significantly mungbean increased yield up to 30%. Rachaputi et al. (2015) demonstrated that optimum row spacing in mungbean grown in subtropical regions resulted in greater intercepted radiation and use of soil water. Proper mungbean density in cropping might reduce competition between species for growth factors such as water, nutrient, and solar radiation (Saleem et al., 2015; Sabbagh and Lakzayi, 2016).

This study reported total LER of all intercropped levels was always greater than monocropping. Partial LER of millet ranged from 0.94 to 1.39, while mungbean partial LER varied from 0.51 to 1.09 in the intercropping systems. Yield advantages of mungbean could be associated with significant variations in mungbean partial LER which generally increased with increasing density of mungbean in millet systems. Thus, intercropping millet with mungbean can result in a yield advantage and greater land use efficiency compared to millet grown alone. Mohammed et al. (2008) and Lithourgidis et al. (2011) reported that increasing legume proportion in intercropped systems with cereals significantly increased total LER due greater yield advantage. The yield advantage of millet-mungbean intercropping over sole millet has been also reported by Trail et al. (2016) in Senegal, Gong et al. (2019) in China, Ghilotia et al. (2015) and Ram and Meena (2015) in India. Sarr et al. (2008) and Diangar et al. (2004) reported LER of 1.68 and 1.37 under millet-cowpea intercropping, respectively, and concluded that intercropping systems can use less land area and produce more grain than monocropped millet in Senegal.

Intercropping systems significantly affected the estimates of millet and mungbean canopy cover. Intercropping increased canopy cover compared to monocropped millet. Similarly, Trail et al. (2016) reported that millet grown alone had lower ground cover compared to intercropped

millet with mungbean. In contrast, Biriah et al. (2017) studied the effects of maize-cowpea intercropping on ground cover and reported greater percent ground cover (74% on average) for cowpea alone compared to maize grown alone and in association. The higher canopy cover reported for millet-164% mungbean (T₈) in our study could be attributed to increased plant density. In addition, increased canopy cover with increasing mungbean density could be due to the linear growth pattern of legume crops. Similar results were observed by Lithourgidis et al. (2011) who reported a quadratic growth for cereals while legumes showed a linear pattern. Higher coverage rate for T₈ could reduce water evaporation and thus improve water use efficiency (Ogindo and Walker, 2005). Treatments with the highest mungbean density had the lowest weed density compared to millet grown alone (Diatta, unpublished data, 2018). Reduction of pest/weed incidence was also observed in intercropping systems with high coverage compared to monocropping (Daryanto et al., 2020). This result could be attributed to the creation of adverse weed germination conditions such as light interception by associated crops (Saha, 2017). Other benefits from intercropping systems with high cover rates include control soil erosion by reducing surface runoff (Seran and Brintha, 2010).

Normalized difference vegetation index (NDVI) values were estimated over the growing season to detect N nutritional differences between millet monocrop and intercropped with mungbean. Millet grown with mungbean had significantly higher NDVI values than millet grown alone and tended to vary at different growth stages. Among intercropping treatments, NDVI values were highest for mungbean grown at the highest densities as reported by Erdle et al. (2011). Trail et al. (2016) found a 9% increase of NDVI on average for millet-mungbean intercropping compared to millet monocropping in Senegal. Similar results were observed in Senegal by Bogie et al. (2019). Conversely, the study conducted by Klimek-Kopyra et al. (2018)

revealed that a legume grown alone had a significantly higher NDVI values (up to 25%) than intercropped legume at developmental stages. In our study, an increase in NDVI values under intercropping can be attributed to increased availability of symbiotically fixed N which can be transferred from mungbean to millet plants (Thilakarathna et al., 2016). The glasshouse experiment conducted by Diatta et al. (unpublished data, 2019) to assess symbiotic performance of mungbean using the ^{15}N natural abundance method revealed that mungbean can fixed up to 75 mg plant⁻¹. However, research on nitrogen fixation by mungbean under field conditions is needed to assess its N₂ fixation potential and contribution to cropping systems. Shah et al. (2003) reported that mungbean can fix up to 110 kg N ha⁻¹, while Senaratne et al. (1995) found that maize grown with mungbean in intercropping systems could derive 7-11% of its N from the N fixed by mungbean. Li et al. (2009) revealed that formation of arbuscular mycorrhizas was enhanced by mungbean and rice intercropping, which in turn increased N transfer (up to 16%) from mungbean to rice plants.

Conclusion

Millet-mungbean intercropping systems can be promoted as a sustainable strategy for increasing yields in low-input agriculture and/or high-risk environment in the semi-arid regions of Senegal. This study aimed at evaluating the productivity of millet-mungbean intercropping system in the semi-arid regions of Senegal. Among intercropping systems, millet + 62% mungbean (T₆) and millet + 125% mungbean (T₇) recorded the highest combined grain yields compared to other treatments in Bambey and Nioro, respectively. This field experiment also revealed that intercropping systems significantly increased LER as a result of increased combined grain yields. LER data revealed that T₆ and T₇ increased mungbean yield without decreasing millet yield and thus was found to be more advantageous than millet monocropping

and mungbean systems in the semi-arid regions of Senegal. Intercropping significantly increased ground canopy cover and NDVI values in millet systems. Treatments T₇ and millet + 164% mungbean (T₈) had higher canopy cover than millet and mungbean alone. Overall, the optimum mungbean plant density for increasing combined yield was T₆ in the West Central Agricultural Region and T₇ in the Saloum Agricultural Region of Senegal. These results demonstrated that intercropping millet with mungbean can improve the productivity of millet and increase its LER, canopy cover estimates, and NDVI values.

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Chapter 5 – Nitrogen fixation by mungbean genotypes in different soil textures

Abstract

Ensuring food and nutritional security in light of high climate variability and a rapidly growing population remains a challenge. Mungbean [*Vigna radiata* (L.) Wilczek] is a short duration and drought-tolerant legume crop capable of symbiotic atmospheric nitrogen fixation. Estimates of biological N₂ fixation by mungbean in different soil textures has not been extensively studied. We conducted this study to evaluate plant growth and N₂ fixation of five mungbean genotypes inoculated with *Bradyrhizobium* and grown in loamy sand and silt loam soils under glasshouse conditions. The percent of nitrogen derived from atmosphere (%Ndfa) by mungbean genotypes using the ¹⁵N natural abundance method was lower in the silt loam soil than the loamy sand soil. As a result, mungbean took up more mineral N from the silt loam soil (235 mg plant⁻¹) than loamy sand soil (40 mg plant⁻¹). Mungbean also fixed more N when grown in the silt loam soil (67 mg plant⁻¹) than on loamy sand (16 mg plant⁻¹), demonstrating mungbean relied more on soil mineral N than symbiotically fixed N for its N nutrition. Among genotypes, IC 8972-1 had the highest biomass (7.85 g plant⁻¹), shoot N content (200 mg plant⁻¹), and soil N uptake (155 mg plant⁻¹) than other genotypes. The significant interaction between soil texture and genotypes for root dry matter and %Ndfa indicates the major role of legume root-nodule bacteria in symbiotic N₂ fixation. Correlations between dry matter and N-fixed, soil N uptake and δ¹⁵N (‰), and shoot N content were significant, confirming that N₂ fixation in mungbean is affected by both genotype and soil properties. This study demonstrated that N₂ fixation by mungbean genotypes is influenced by soil properties.

Keywords: Mungbean, Nitrogen fixation, %Ndfa, Soil texture

Introduction

In symbiosis with rhizobia, legumes can fix atmospheric nitrogen and contribute to meeting nitrogen needs in agriculture. N₂ fixation plays an important role in increasing crop N-use efficiency and reducing N losses to the environment (Maseko et al., 2020). Biological fixation of atmospheric N₂ by legumes corresponds to its conversion by N₂-fixing bacteria called diazotrophs into plant-available ammonia (NH₃) using nitrogenase enzymes (Unkovich et al., 2008). This ability makes legume crops a viable option for providing N to the companion/subsequent crops and for developing more sustainable production systems (Peoples et al., 1995; Foyer et al., 2016; Diatta et al., 2019b). Annual estimates of N fixed by legumes in symbiosis with soil rhizobia were reported to be 21.5 Tg (Herridge et al., 2008) of which up to 30% is returned to soils (Reeves et al., 2016). Other reported benefits of including legumes into crop rotations are greater microbial and crop diversity, soil fertility, and reduction of pest incidence (Giller, 2001; Matusso et al., 2014; Franke et al., 2018; Diatta et al., 2019a; Vanlauwe et al., 2019; Singh et al., 2020). Legume crops are an important and economic source of protein, minerals, and vitamins in human diets and animal feed under smallholder agriculture (Graham and Vance, 2003; Tharanathan and Mahadevamma, 2003). To optimize legume crop potential for promoting productive and sustainable cropping systems, the contribution of legumes to agricultural systems need to be assessed to enable maximization of symbiotic N₂ fixation (Thilakarathna et al., 2016; Vanlauwe et al., 2019).

Mungbean is a short-duration legume crop widely grown in arid and semiarid environments of the tropics. It is cultivated on over 7 million ha and has recently experienced yield increases due to the development of varieties with short and synchronous maturity, higher yield, and greater disease-resistance (Nair et al., 2019). Potential yields of 2.5-3.0 Mg ha⁻¹ have been reported in mungbean, but lower yields (0.5 Mg ha⁻¹) have been also observed, particularly in smallholder

cropping systems (Pandey et al., 2018; Diatta et al., 2019a). Mungbean fits well into many cropping systems because of its short growth cycle and low water and N requirements (Chadha, 2001; Diatta et al., 2019a). In mungbean, fixed N ranged from 6 to 112 kg ha⁻¹ (Shah et al., 2003; Ali et al., 2013a), allowing the crop to meet up to 75% of its N requirements from biological N₂ fixation (Shah et al., 2003). Dakora et al. (2015) reported that mungbean could fix up to 110 kg N ha⁻¹, while Zang et al. (2015) observed that 10% of fixed N can be transferred to companion and subsequent crops. Thus, it represents an opportunity to produce highly nutritious food under arid and semiarid conditions particularly for smallholder farmers (Keatinge et al., 2011). Limited production in regions other than South and Southeast Asia, Australia, and East Africa (Keatinge et al., 2011; Diatta et al., 2018) has resulted in inadequate research to understand the observed variation in N₂ fixation by mungbean and assess its contribution to cropping system (Herridge et al., 2008; Thilakarathna et al., 2016; Raji et al., 2019; Vanlauwe et al., 2019).

N₂-fixation capacity of legumes is influenced by several factors including inherent genetic potential and the presence of compatible and sufficient rhizobial communities in the soil (Franke et al., 2018). As a result, Herridge et al. (2005) recommended the selection of responsive soils and plants that are symbiotically competent and use of rhizobial strains present in the soil to increase nodulation and N₂ fixation by mungbean. Other parameters also affecting plant growth such as water deficit, nutrient availability particularly N and phosphorous, light and temperature, and insect-pests and disease incidence can influence the rates of fixed N (Diatta et al., 2019b; Nair et al., 2019; Vanlauwe et al., 2019; Singh et al., 2020). Furthermore, symbiotic performance of legumes can be affected by plant density, species, and agronomic practices (Makoi et al., 2009).

Soil texture is known to have a significant effect on *Rhizobium* survival, root development, crop growth, and biomass yield of mungbean which subsequently affect N₂ fixation yields

(Kirchhof and So, 1995; Singh et al., 2011; Diatta et al., 2018; Diatta et al., 2019b). Ahmad et al. (2015) demonstrated that a suitable soil can increase water and nutrient uptake by mungbean due to better root development. Other researchers have reported that beneficial effects include good aeration and drainage, soil water storage capacity, and nutrient retention (Travlos and Karamanos, 2006; Zhao et al., 2015; Ntukamazina et al., 2017). In addition to soil texture, N₂-fixing ability of mungbean has been demonstrated to vary by genotype (Delfin et al., 2008). Razzaque et al. (2016) showed that nodulation and biologically fixed N differed among 10 mungbean genotypes. Others have also reported variations in N₂ fixation rates due to inherent genotypic variability (Devi et al., 2010; Devi et al., 2013).

Most N fixation studies have focused on major crop legumes such as soybean, groundnut, cowpea, common bean, and chickpea. In contrast, estimates of biological N₂ fixation by mungbean are still lacking (Raji et al., 2019). A study conducted by Shah et al. (2003) in Pakistan revealed that 54 to 82% of mungbean N requirements can be met from 55 to 86 kg N ha⁻¹ of fixed N. However, Hayat et al. (2008) reported lower values ranging from 19 and 47 kg N ha⁻¹ and representing between 32% to 49% of mungbean total N nutrition. In the Philippines, George et al. (1995) reported that N fixed by mungbean ranged from 61 to 90 kg N ha⁻¹ while Rosales et al. (1995) observed 21 to 85 kg N ha⁻¹. Variations in N₂ fixation estimates highlight the need to investigate these factors to better maximize N contribution to cropping systems. Of the limited research conducted on mungbean, most studies examined the effects of *Bradyrhizobium* inoculation, tillage, crop residues management, and mungbean-cereal sequences on N₂ fixation (Delfin et al., 2008; Hayat and Ali, 2010; Mohammad et al., 2010; Ali et al., 2013b). However, little research has focused on the combined effects of soil texture and genotype on N₂ fixation potential. The aim of this study was to assess plant dry matter production and N₂ fixation of

mungbean genotypes grown under greenhouse conditions in silt loam and loamy sand soils using the ^{15}N natural abundance method.

Materials and methods

Soil description

Study soils were collected from Virginia Tech's Tidewater Agricultural Research and Extension Center (AREC) in Suffolk, VA (36°39'50.9"N latitude, 76°44'01.0"W longitude) and Kentland Farm in Blacksburg, VA (37°11'47.3"N latitude, 80°34'49.7"W longitude), respectively. The site in Suffolk has a Eunola loamy fine sand (Fine-loamy, siliceous, semiactive, thermic Aquic Hapludults) and had been under a corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) rotation, while the soil in Blacksburg was characterized by a continuous corn system and is a Guernsey silt loam (Fine, mixed, superactive, mesic Aquic Hapludalfs) (NRCS, 2019).

Soil sampling and analysis

Soils were collected by excavating to a depth of 20 cm from fields with no recent history of legumes in the crop management systems at those sites. After collection, composite soil samples were placed in plastic containers and air-dried in a dust-free glasshouse and then sieved to pass a 5 mm particle size diameter. Field capacity and soil pH, and plant available Ca, Mg, P, and K of soil samples were determined as described by Gupta and Larson (1979) and Maguire and Heckendorn (2011), respectively. The concentrations of (NH_4^+) and nitrate (NO_3^-) were measured using Lachat QuikChem AE flow-injection autoanalyzer and ion chromatography and were prepared as described by Bremner and Keeney (1966). Soil samples were analyzed by dry combustion using a vario MAX CNS Element Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) to determine total C and N. After determining physical and chemical properties (Table 1) of the two soil types, plastic pots (19 cm tall, 19 cm outside diameter, and 3785 cm³

volume) were filled with 3.5 kg of each one of the two soils, which were collected in the field without mixing the different soil horizons. To prevent loss of water and the N-free Hoagland's solution used as fertilizer in this experiment, each plastic pot was lined with a polythene bag.

Table 1. Physical and chemical properties of the Eunola soil and Guernsey soil in the mungbean N₂ fixation study.

| Soil properties † | Eunola soil | Guernsey soil |
|---|-------------|---------------|
| Soil texture | Loamy sand | Silt loam |
| Field capacity (%) | 9 | 17 |
| Soil pH | 5.9 | 6.2 |
| C (%) | 0.98 | 0.93 |
| N (%) | 0.08 | 0.08 |
| C/N | 13 | 11 |
| NO ₃ ⁻ (mg kg ⁻¹) | 1.52 | 2.67 |
| NH ₄ ⁺ (mg kg ⁻¹) | 0.32 | 0.25 |
| P plant available (mg kg ⁻¹) | 37 | 26 |
| K plant available (mg kg ⁻¹) | 63 | 61 |
| Ca plant available (mg kg ⁻¹) | 575 | 859 |
| Mg plant available (mg kg ⁻¹) | 72 | 167 |

† pH: soil (1:1 soil: deionized water mixture on a volumetric basis); Total carbon (C) and total nitrogen (N): vario MAX CNS Element Analyzer; Inorganic NO₃⁻ and NH₄⁺: 2M KCl extraction; Plant available nutrients: soil (Mehlich 1 solution).

Experimental design and planting

Two factors were used in this greenhouse experiment. Mungbean genotypes were composed of one hybrid (Berken) (PI 662968, *Vigna radiata*) and 4 open-pollinated [8735 (PI 164442; *Vigna radiata* var. *radiata*), IC 8972-1 (PI 363217; *Vigna radiata* var. *radiata*), STB#122 (PI 425083; *Vigna radiata* var. *radiata*), and 223 (PI 473850; *Vigna radiata*)] (i.e., ‘genotypes’; 5

levels). The second factor (i.e., ‘soil texture’) had two levels: loamy sand and silt loam textures. Berken is a hybrid, medium-large seeded, and grown cultivar in the U.S. and was obtained from Oklahoma Foundation Seed Stocks (Stillwater, OK, USA). Open-pollinated mungbean seeds were obtained from the U.S. National Plant Germplasm System. Before sowing, mungbean seeds were inoculated with *Bradyrhizobium* inoculum (group I) at a rate of 10 g inoculant kg⁻¹ seed. The seed inoculant contained less than 1% of active ingredients [*Bradyrhizobium* sp. (*Vigna*), *Bradyrhizobium japonicum*, *Rhizobium leguminosarum biovar phaseoli*, *Rhizobium leguminosarum biovar viceae*] by weight and 99% of inactive ingredients. Sorghum [*Sorghum bicolor* (L.)] and wheat (*Triticum aestivum*) were selected as the non-N₂ fixing reference plants. Seeds of sorghum were obtained from the Tidewater Agricultural Research and Extension Center (AREC) while those of wheat were provided by the wheat breeding group at Virginia Tech. Mungbean and non-legume species were each sown in two soil textures on May 31, 2019 at the Virginia Tech’s greenhouse and maintained at 80% field capacity. Field capacity was determined at –0.33 bar water potential using pressure chambers in a 1500F2, 15 bar pressure plate extractors (Soil moisture equipment corp., Santa Barbara, CA). Temperature control systems in the glasshouse were thermostatically controlled keep the minimum daytime temperature at 29°C for 10 h and the minimum night-time temperature at 25°C for 14 h. The experimental pots were laid out in a randomized complete block design with factorial arrangement of factor-levels, resulting in 10 treatment combinations that were replicated six times each.

Plant sampling and processing

At the pod-filling stage (48 days after planting – DAP), mungbean was harvested and separated into shoots and roots while only shoots of reference plants were collected. Plant samples were oven-dried for 48 h at 60°C to constant weight and weighed separately to determine dry

matter yield. Plants were then ground to <0.25 mm particle size and placed in 1.5 mL polyallomer tubes (Beckman Instruments, Inc., Fullerton, CA). Prior to shipping for ¹⁵N isotopic analysis, finely milled samples were weighed to 3 mg using a Mettler Toledo UMX2 Automated Ultra-Micro Balance (Huntsville, AL) accurate to 0.001 mg, stored into 5x5 mm tin capsules, and placed in 96-well microtiter plates.

Determination of biological N₂ fixation

The ¹⁵N natural abundance technique exploits naturally occurring differences in ¹⁵N composition between plant-available N sources in the soil and that of atmospheric N₂ (Robinson, 2001; Unkovich et al., 2008). The ¹⁵N natural abundance and total N of mungbean and reference plant samples were quantified using the Costech Elemental Analyzer (EA) interfaced to Finnigan MAT-252 isotope ratio mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The ¹⁵N abundance is the ‰ deviation of the relative abundance of the sample isotopes from the international standard ($atom\%^{15}N_{atmosphere} = 0.3667$) defined by International Atomic Energy Agency (IAEA). It is expressed using delta (δ) notation (Unkovich et al., 1994).

$$\delta N^{15}(\text{‰}) = \frac{atom\%^{15}N_{sample} - atom\%^{15}N_{atmosphere}}{atom\%^{15}N_{atmosphere}} \times 1000$$

where $atom\%^{15}N_{sample}$ is the percentage of isotopic abundances ratios of sample

(¹⁵N/ ¹⁴N) while $atom\%^{15}N_{atmosphere}$ is the percentage isotope ratio (¹⁵N/ ¹⁴N) in standard air.

The percent N derived from the atmosphere (%Ndfa) in mungbean was estimated as described by (Shearer and Kohl, 1986; Unkovich et al., 2008), as follows:

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

where $\delta^{15}N_{reference\ plant}$ is the ^{15}N natural abundance of non- N_2 fixing reference plant obtaining all its N from the soil. $\delta^{15}N_{legume}$ is the ^{15}N natural abundance of mungbean deriving all N from symbiotic N_2 fixation. The B value used for mungbean was -2.50‰ and is defined as $\delta^{15}N$ of mungbean shoots only dependent on symbiotic N_2 fixation for N nutrition (Shah et al., 2003; Unkovich et al., 2008). The proportion of N derived from atmosphere in mungbean was quantified using mean $\delta^{15}N_{reference\ plant}$ of reference plants. The total N content in mungbean shoots was determined by multiplying mungbean %N and shoot dry biomass weight. The amount of N-fixed was calculated as the product of the percentage of N_2 fixation derived from the atmosphere and the total N content of mungbean shoots (Unkovich et al., 2008). The amount of soil N uptake from soil was obtained by the difference between plant total N and N-fixed.

$\delta^{15}N$ values of reference plant species

To determine the proportion of N derived from the atmosphere (%Ndfa), $\delta^{15}N$ values from shoots of non-fixing legume plants (i.e., sorghum and wheat) were sampled, processed, and analyzed similarly to mungbean. The $\delta^{15}N$ of sorghum shoots in the loamy sand and the silt loam soils were 4.56‰ and 9.25‰ , respectively (Table 2). Mean of $\delta^{15}N$ values for sorghum under different soil textures was 6.91‰ . An average value of 7.07‰ was recorded for $\delta^{15}N$ in wheat from loamy soil (4.66‰) and silt loam (9.48‰) soils. The $\delta^{15}N$ values of reference plants were always lower in loamy sand compared to silt loam, suggesting that $\delta^{15}N$ values might be influenced by soil texture (Table 2). Differences in background $\delta^{15}N$ values between soil textures as a result of different soil mineral N have also reported by other authors (Mokgehle et al., 2014; Beyan et al., 2018; Raji et al., 2019).

Table 2. $\delta^{15}\text{N}$ (‰) of reference plants used for estimating soil N uptake by mungbean genotypes in loamy sand and silt loam soils.

| Reference plants | Loamy sand | Silt loam |
|-----------------------------|----------------------------------|------------|
| | $\delta^{15}\text{N}$ values (‰) | |
| Sorghum | 4.56±0.64b† | 9.25±0.23a |
| <i>Sorghum bicolor</i> (L.) | | |
| Wheat | 4.66±0.63b | 9.48±0.06a |
| <i>Triticum aestivum</i> | | |
| Average | 4.61±0.43b | 9.37±0.12a |

† Values (Mean ± SE) with dissimilar letters in a row are significantly different at $\alpha = 0.05$.

Samples of non-fixing legumes were replicated six times.

Statistical and correlation analyses

Statistical analysis was performed on mungbean dry matter, shoot N concentration and content, $\delta^{15}\text{N}$, %Ndfa, N-fixed, and soil N uptake using JMP Pro version 15 statistical software (SAS Institute Inc., Carey, NC). Analysis of variance (ANOVA) was used to test the effects of mungbean genotypes and soil texture on plant growth and symbiotic performance parameters. When treatment effects were significant at $\alpha = 0.05$, Fisher's protected LSD was used for mean separation. Correlation analyses were performed to ascertain the relationships between N fixed and mungbean dry weight as well as soil N uptake and shoot $\delta^{15}\text{N}$ and soil N uptake and Ndfa %.

Results

Dry matter yield of mungbean

There was a significant soil texture x genotype interaction for dry matter of mungbean roots but not for shoot and whole-plant dry matter (Table 3), indicating that genotypes had different root growth responses in the two soil textures. Analysis of root dry matter production showed that IC 8972-1 (1.4 g plant⁻¹) and Berken (1.43 g plant⁻¹) genotypes produced greater biomass than STB #122, 223, and 8735 genotypes in the loamy sand soil (Table 4). Similarly, genotypes 223 (2.78 g plant⁻¹) and STB #122 (2.59 g plant⁻¹) produced more root biomass in silt loam soil compared to other genotypes (Table 4).

There were significant differences ($P < 0.05$) for plant growth (shoot and whole-plant dry matter) of mungbean grown in different soil textures (Table 3). Mungbean grown in silt loam soil produced significantly higher shoot (7.72 g plant⁻¹) biomass than plants in sandy loam soil (2.83 g plant⁻¹) (Table 5). As a result, whole-plant dry matter in silt loam soil (10 g plant⁻¹) was 151% higher than total biomass (4 g plant⁻¹) (Table 5). Genotype IC 8972-1 (6.04 g plant⁻¹) produced significantly greater shoot dry matter, which was not different from STB #122. The lowest shoot biomass was recorded for genotype 8735 (4.72 g plant⁻¹) (Table 5). Similar to shoot dry matter, significant ($P < 0.05$) differences were also observed for total-plant dry matter yield between mungbean genotypes which followed the same pattern. The lowest biomass was produced by 8735 (6.11 g plant⁻¹), while biomass measured for genotype IC 8972-1 (7.85 g plant⁻¹) and STB #122 (7.37 g plant⁻¹) was greatest (Table 5).

$\delta^{15}\text{N}$ and %Ndfa values

The interaction of soil texture and genotypes was significant for %Ndfa, but not for $\delta^{15}\text{N}$ values (Table 3), indicating that %Ndfa of mungbean genotypes varied significantly under the two soil textures. Comparison of %Ndfa values of mungbean genotypes in the two soil textures revealed significant differences only in the sandy loam soil (Table 4). Genotype 223 derived more N from the atmosphere (42.9%) in the loamy sand soil which was not different from STB #122 (31.2%), the lowest values were recorded for 8735, IC 8972-1, and Berken (Fig. 2A). However, there were no significant differences ($P>0.05$) among mungbean genotypes for %Ndfa values in silt loam soil (Table 4).

The mean of $\delta^{15}\text{N}$ values of mungbean grown in two soil textures were significantly different ($P<0.05$) (Table 3). Plants grown in silt loam soil recorded mean of $\delta^{15}\text{N}$ values of 9.12‰ while mungbean genotypes in a loamy sand were 4.02‰ (Table 5). However, mean $\delta^{15}\text{N}$ values did not differ between mungbean genotypes (Table 5).

Shoot N content, amount of N-fixed and Soil N uptake

Shoot N content of mungbean differed ($P<0.05$) between the two soil textures (Table 3). Mungbean plants in sandy loam soil recorded 432% lower N content in shoots compared to mungbean grown in silt loam ($303.4 \text{ mg plant}^{-1}$) (Table 5). The N content of mungbean shoots also significantly varied between the five genotypes. Genotype IC 8972-1, which had the highest whole-plant dry matter, recorded the highest N content ($200 \text{ mg plant}^{-1}$), while 8735 exhibited the lowest N content of $160 \text{ mg plant}^{-1}$ (Table 5). Overall, genotype IC 8972-1 always recorded the highest biomass and shoot N content while 8735 always recorded the lowest values. The similar pattern for whole-plant dry matter and N content might indicate that N content in mungbean genotypes might be influenced by dry biomass.

The amount of N-fixed by mungbean in silt loam soil was 67 mg plant⁻¹ and was significantly higher ($P < 0.05$) than plants grown in loamy sand soil which fixed 314% less N (Table 3). Similar to shoot N concentration and N content, mungbean plants in silt loam soils which produced the largest shoot biomass also fixed the highest amount of N. Despite significant differences in shoot dry mass between genotypes, the amount of N contribution by mungbean ranged insignificantly from 36.7 to 45.5 mg plant⁻¹, indicating the significant role of soil properties on the efficiency of N₂ fixation in mungbean (Table 5).

As the case with symbiotically fixed N, mungbean in silt loam soil derived more N from the soil than plants in loamy sand soil (Table 5). Genotypes in loamy sand soil obtained 40.8 mg plant⁻¹ while, mungbean plants in silt loam soil had 235 mg plant⁻¹ from the soil. A comparison of N-fixed and soil N uptake by soil texture revealed that mungbean took up 151% in loamy soil and 249% in silt loam more N from soil than from symbiotic N₂ fixation to meet their N requirements (Table 5). Higher soil N uptake in silt loam soil is explained by the relatively higher concentrations of nitrogen (59% more) compared to the loamy sand soil (Table 1). Overall, mungbean genotypes fixing more N symbiotically also took up more N from the soil. The mean of soil N uptake ranged from 123 to 155 mg plant⁻¹ and significantly varied between mungbean genotypes. As shown in Table 3, genotype IC 8972-1 obtained 155 mg plant⁻¹ of N the from the soil, followed by STB #122, Berken, 223, while 8735 took up the lowest amount of N (123 mg plant⁻¹) (Table 5).

Table 3. Two-way ANOVA of dry matter (DM) yield in shoot, root and whole plant, percent of nitrogen derived from atmosphere (%Ndfa), N content, concentration and N₂ fixation in shoot, and soil N uptake of mungbean genotypes in a loamy sand and silt loam soils.

| | df | DM Shoot† | DM Root | DM Plant | $\delta^{15}\text{N}$ | Ndfa | Shoot N conc. | Shoot N content | N-fixed | Soil N-uptake |
|---------------------------|----|-----------------------|---------|----------|-----------------------|------|------------------------|-----------------|---------|---------------|
| | | g plant ⁻¹ | | | % | % | mg plant ⁻¹ | | | |
| Soil texture | 1 | *** | *** | *** | *** | ** | *** | *** | *** | *** |
| Genotypes | 4 | *** | * | *** | ns | * | ns | ** | ns | * |
| Soil texture x Genotypes‡ | 4 | ns | * | ns | ns | ** | ns | ns | ns | ns |
| Model | 9 | | | | | | | | | |
| Error | 50 | | | | | | | | | |
| Total | 59 | | | | | | | | | |

† Mean values are significantly different at $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), or ns = not significant.

‡ The interaction between soil texture and genotypes was significant for DM root and Ndfa and data is presented in Table 5.

Table 4. Dry matter root and %Ndfa values of mungbean genotypes in a loamy sand and silt loam soils.

| Genotypes | DM Root | | %Ndfa | |
|-----------|-----------------------|---------------|----------------|---------------|
| | Loamy sand | Silt loam | Loamy sand | Silt loam |
| | g plant ⁻¹ | | % | |
| 8735 | 0.83 ± 0.18b | 1.96 ± 0.20c | 23.61 ± 2.94b | 22.23 ± 2.70a |
| IC 8972-1 | 1.4 ± 0.13a | 2.23 ± 0.21bc | 22.49 ± 3.50b | 22.97 ± 1.22a |
| STB #122 | 1.1 ± 0.07ab | 2.59 ± 0.13ab | 31.23 ± 5.28ab | 22.40 ± 2.02a |
| 223 | 1.08 ± 0.10ab | 2.78 ± 0.17a | 42.85 ± 5.46a | 20.45 ± 2.91a |
| Berken | 1.43 ± 0.19a | 2.05 ± 0.22bc | 20.91 ± 3.88b | 23.49 ± 2.35a |

Values (Mean S.E.) with dissimilar letters in a column are significantly different at $\alpha = 0.05$.

Table 5. Shoot and whole-plant dry matter yield, $\delta^{15}\text{N}$, shoot N concentration and content, N-fixed, and soil N uptake of mungbean genotypes in a loamy sand and silt loam soils.

| | DM Shoot | DM Plant | $\delta^{15}\text{N}$ | Shoot N conc. | Shoot N content | N-fixed | Soil N-uptake |
|---------------------|-----------------------|---------------|-----------------------|---------------|------------------------|--------------|------------------|
| | g plant ⁻¹ | | ‰ | % | mg plant ⁻¹ | | |
| Soil texture | | | | | | | |
| Loamy sand | 2.83 ± 0.11b† | 4.00 ± 0.15b | 4.02 ± 0.05b | 2.03 ± 0.07b | 57.06 ± 3.0b | 16.25 ± 1.5b | 40.81 ± 2.6b |
| Silt loam | 7.72 ± 0.17a | 10.04 ± 0.21a | 9.12 ± 0.08a | 3.93 ± 0.04a | 303.44 ± 7.3a | 67.25 ± 3.2a | 235 ± 6.7a |
| Genotypes | | | | | | | |
| 8735 | 4.72 ± 0.69c | 6.11 ± 0.87c | 6.62 ± 0.76a | 3.00 ± 0.27a | 159.98 ± 32.6c | 36.74 ± 8.6a | 123.24 ± 25.0c |
| IC 8972-1 | 6.04 ± 0.78a | 7.85 ± 0.92a | 6.60 ± 0.75a | 2.88 ± 0.32a | 200.42 ± 41.5a | 45.30 ± 9.4a | 155.11 ± 32.5a |
| STB #122 | 5.53 ± 0.81ab | 7.37 ± 1.03ab | 6.53 ± 0.78a | 3.00 ± 0.32a | 191.64 ± 41.0ab | 45.49 ± 8.5a | 146.14 ± 33.3ab |
| 223 | 4.92 ± 0.72c | 6.84 ± 0.97b | 6.50 ± 0.85a | 3.10 ± 0.31a | 173.99 ± 36.6bc | 41.25 ± 6.7a | 132.74 ± 31.7bc |
| Berken | 5.18 ± 0.79bc | 6.92 ± 0.90b | 6.60 ± 0.74a | 2.92 ± 0.29a | 175.22 ± 37.4bc | 40.70 ± 9.8a | 134.52 ± 28.6abc |

† Values (Mean ± SE) with dissimilar letters in a column are significantly different at $\alpha = 0.05$.

Correlation analysis

Correlation analyses were performed to evaluate if soil N uptake in the loamy sand and silt loam soils altered shoot dry matter, $\delta^{15}\text{N}$ (‰), %Ndfa, and shoot N content. The results in Fig. 1 show a significantly positive relationship between shoot dry matter and soil N uptake in the loamy sand ($r = 0.71^{***}$) and silt loam soil ($r = 0.76^{***}$). There was also a significantly positive association between $\delta^{15}\text{N}$ (‰) and soil N uptake in both soils (Fig. 2). As a result, we observed a significantly negative relationship between %Ndfa and soil N uptake by mungbean genotypes (Fig 3). To evaluate whether, increasing soil N uptake resulted in higher shoot N content, we measured the correlation coefficient and noted a significant relationship of $r = 0.85^{***}$ and $r = 0.90^{***}$ in the loamy sand (Fig 4A) and silt loam soil (Fig 4B), respectively. These results revealed that an increase in soil N uptake induced an increase in shoot dry matter, shoot N content, and $\delta^{15}\text{N}$ values, which lowered %Ndfa values (Figs. 1, 2, 3, and 4).

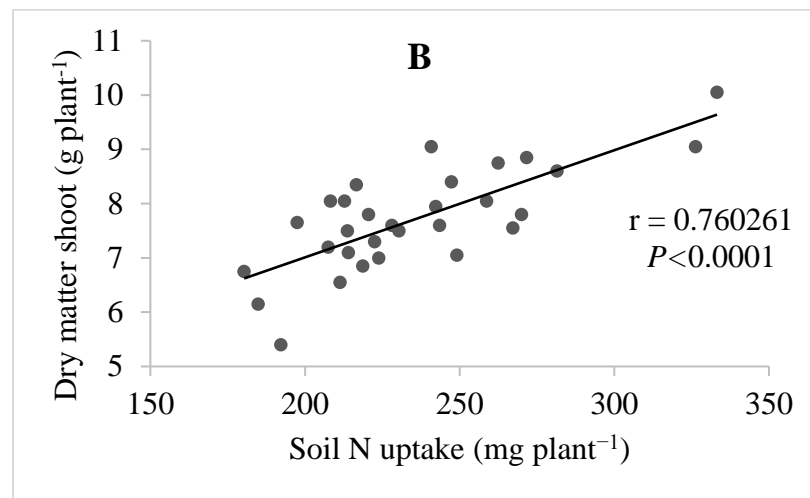
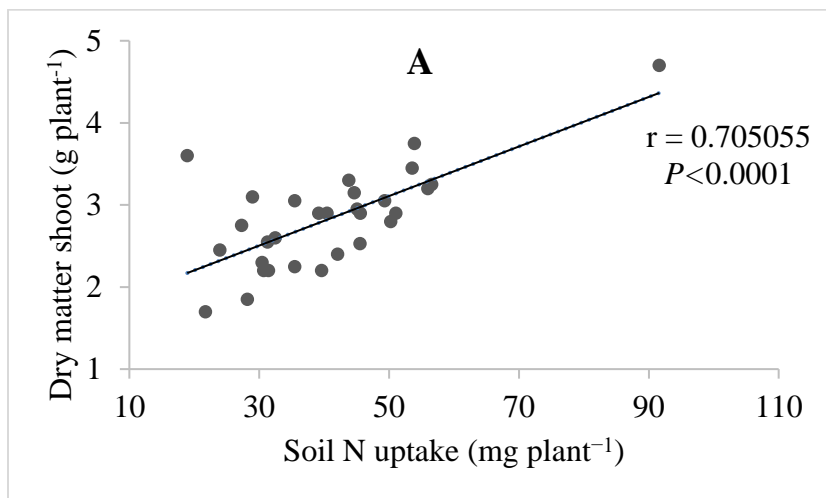


Figure 1. Relationship between shoot dry matter and soil N uptake of mungbean in loamy sand (A) and silt loam (B) soils.

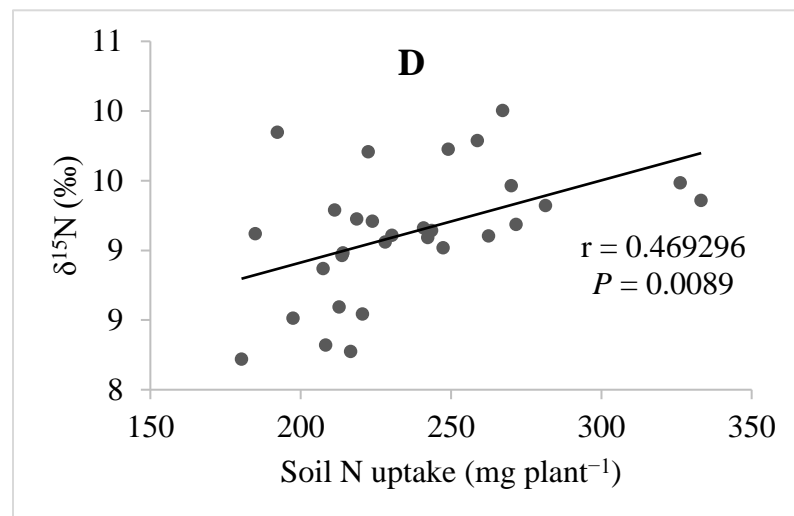
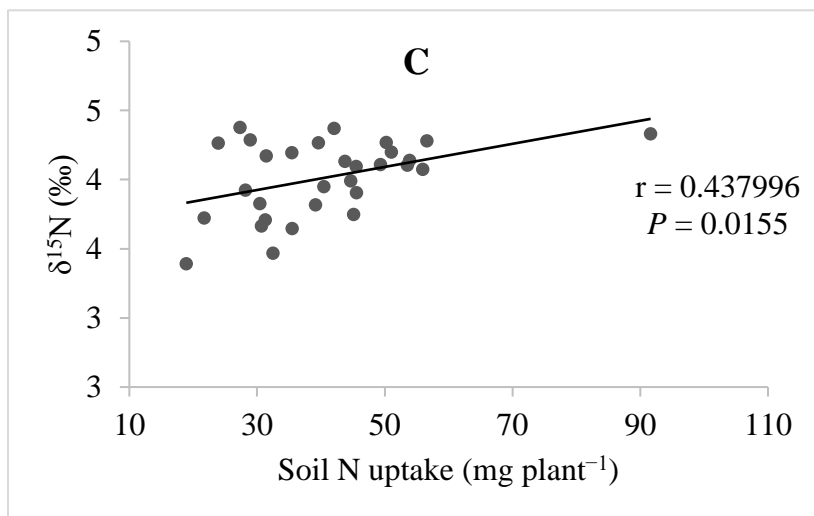


Figure 2. Relationship between δ¹⁵N values and soil N uptake of mungbean in loamy sand (C) and silt loam (D) soils.

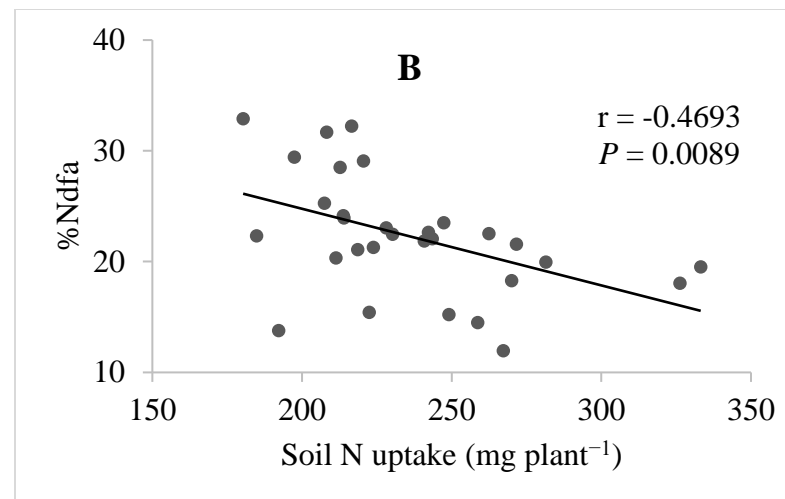
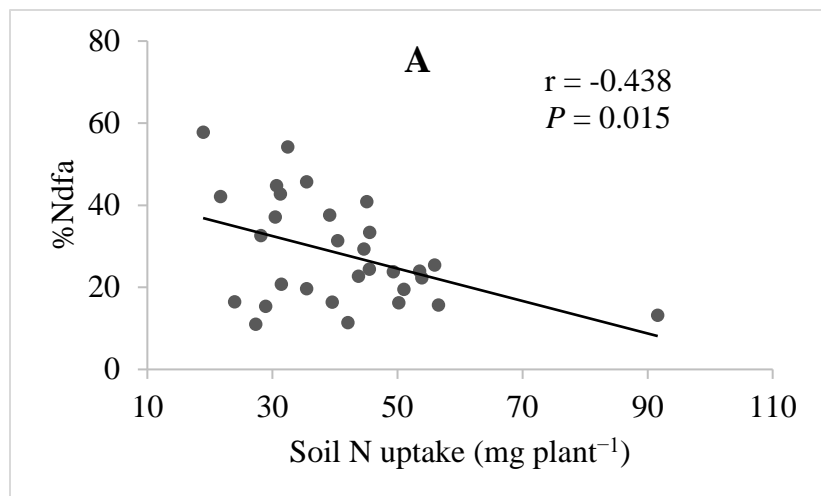


Figure 3. Relationship between %Ndffa and soil N uptake of mungbean in loamy sand (A) and silt loam (B) soils.

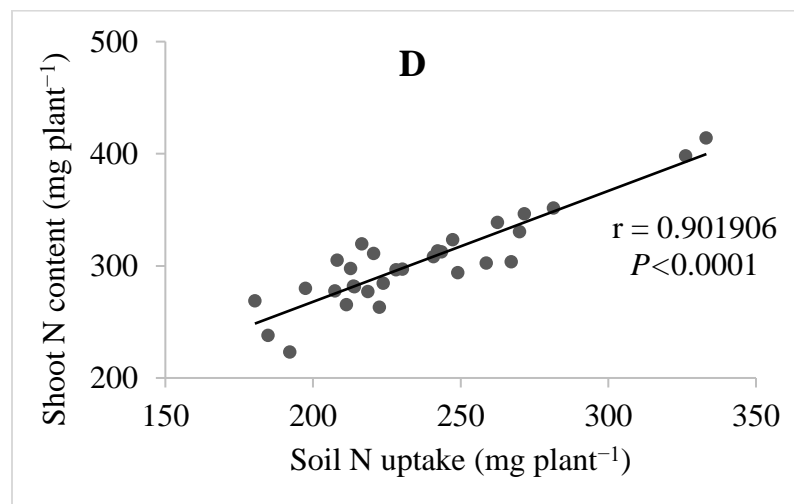
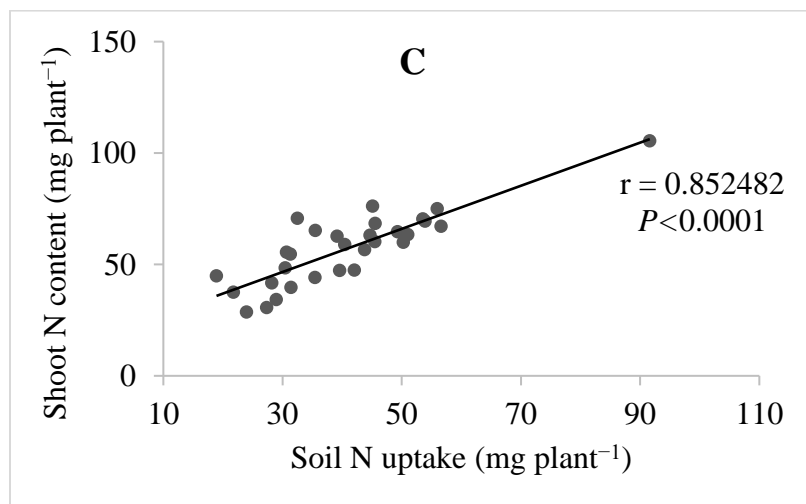


Figure 4. Relationship between shoot N content and soil N uptake of mungbean in loamy sand (C) and silt loam (D) soils.

Discussion

Analysis of the effects of soil texture on mungbean shoot and whole-plant dry matter and shoot N concentration and content revealed significant effects (Table 3). Similarly, $\delta^{15}\text{N}$ values, amount of N-fixed, and soil N uptake of mungbean varied between soil textures, demonstrating soil effects on N_2 fixation (Table 3). Mungbean plants grown in silt loam soil produced more biomass than mungbean grown in loamy sand which corresponded to 100% more dry matter yield than plants in the loamy sand soil (Table 5). Differences in biomass yields are probably due to larger concentrations of soil nutrients in the silt loam soil. Significant differences in mungbean biomass yields grown in different soil conditions have been also reported by Diatta et al. (2018).

Shoot N content of mungbean was 432% more in the silt loam soil than values in the loamy sand soil (Table 5). This result suggests that higher plant available soil N in the silt loam soil might have affected shoot N concentration and content. Shoot $\delta^{15}\text{N}$ values were 127% higher in the silt loam soil than values recorded in the loamy sand soil due to higher concentrations of plant available N. This increase resulted in 27% more N derived from atmosphere in loamy sand compared to silt loam soil (Table 4). Similar results were observed by Herridge et al. (2005) who found that lower availability of soil nitrate resulted in higher %Ndfa values in mungbean. The silt loam soil, despite having higher concentration of soil N, recorded the highest amounts of N_2 fixation ($67.3 \text{ mg plant}^{-1}$) and soil N uptake ($235 \text{ mg plant}^{-1}$). In contrast, Raji et al. (2019) found that higher concentration of N inhibits N_2 fixation in mungbean. Increase in N_2 fixation by mungbean in silt loam soil despite having higher soil N concentration could be attributed to N needs being greater than N supply (Salvagiotti et al., 2008).

Plant growth parameters, shoot N content, and soil N uptake significantly varied between genotypes (Table 3). A recent study (Diatta, unpublished data, 2019) documenting adaptation and performance of mungbean genotypes also reported significant difference for plant growth between 8735, IC 8972-1, STB #122, 223, and Berken genotypes. Similar results were observed on mungbean genotypes by Mbeyagala et al. (2016) in Uganda, Kumar and Sharma (2009) in India, Herridge et al. (2005) in Australia. Variations in plant growth between genotypes could be due attributed to genetic variation (Yimram et al., 2009; Rahim et al., 2010).

Though dry matter yields varied among genotypes, the amount of N fixed was similar, indicating that the amount of N fixed was more influenced by soil texture than genotype. In contrast, Herridge and Rose (2000) reported that higher biomass yield would result in increased N₂ fixation by legume crops. Kermah et al. (2018) assessed N contribution by cowpea, soybean and groundnut and indicated that higher biomass yields resulted in greater amounts of fixed N. In addition, Peoples et al. (2009) concluded that accumulated N in legumes could also affect amount of symbiotically fixed N. The review conducted by Dudeja and Duhan (2005) showed that differences in N₂ fixation among mungbean genotypes are also associated to the efficiency symbiosis with rizophial strains. Soil N uptake differed between mungbean genotypes and ranged from 123 to 155 mg plant⁻¹ for 8735 and IC 8972-1 genotypes, respectively. As for soil texture effects, genotypes with higher soil N uptake rates derived less N from atmospheric N₂. In general, mungbean genotypes took up on average 230% more N from the soil than from symbiosis, and yet fixed up to 45.5 mg plant⁻¹. This could indicate tolerance of mungbean-symbionts to soil

mineral N. Similar findings were observed by Mokgehle et al. (2014) on groundnut and Mapope and Dakora (2016) on soybean in South Africa.

Mungbean genotypes derived different proportions of N from atmosphere when grown in different soil textures. This indicates the controlling effects of soil texture on N₂ fixation potential of mungbean genotypes (Beyan et al., 2018). On average, mungbean genotypes relied more on N from the atmosphere when grown on the loam sand soil than the silt loam soil. Increase of %Ndfa values in the loamy sand soil could be due to the relatively lower fertility status compared to the silt loam soil (Giller, 2001). Beyan et al. (2018) attributed increase in $\delta^{15}\text{N}$ values and subsequent decrease in %Ndfa between soybean genotypes to effective symbiosis with rhizobial populations in soils. Reported %Ndfa values from our study were consistent with the range of 15% and 90% %Ndfa reported by Peoples et al. (2009) and (Peoples et al., 1991), respectively. Shah et al. (2003) indicated from his study in Pakistan that mungbean could derive 100% of its N from N₂ fixation. A more recent study on rhizobial inoculation and P addition effects on mungbean reported mean %Ndfa values ranging from 17 to 50% in high input and low-input soils, respectively (Raji et al., 2019). These values indicate wide variation of mungbean dependence on N from atmosphere.

Generally, the mungbean genotypes taking up the greatest amounts of N also produced the highest dry matter biomass. We found a significantly positive correlation ($P < 0.0001$) between soil N uptake and shoot dry matter (Fig. 1). Previous studies have also demonstrated a significantly positive correlation between soil N uptake and plant biomass in mungbean (Delfin et al., 2008). In both soils, mungbean genotypes met their N requirements more from soil N than symbiotically fixed N. There was a significant positive

correlation between soil N uptake and $\delta^{15}\text{N}$ in the loamy sand ($r = 0.44$) and silt loam ($r = 0.47$) (Fig. 2). Mungbean grown in soil with relatively high levels of plant-available N also relied more on soil N uptake than N_2 fixation in field study in Ethiopia (Raji et al., 2019). However, several authors have indicated that limitations in accurately assessing below-ground of fixed N in roots and N inputs through root exudates and decayed root matter could underestimate the amounts of fixed N by legumes (Unkovich et al., 2008; Mapope and Dakora, 2016). We found that genotypes taking up the most N tended to proportionally derive less N from the atmosphere (Fig. 3). Similar results were reported by Mapope and Dakora (2016) when studying symbiotic fixation of soybean genotypes across farmers' fields in South Africa. The significant negative relationship in the loamy sand ($r = -0.44$) and silt loam ($r = -0.47$) between soil N uptake and %Ndfa as shown in Fig. 3 could help explain the important effect of soil N in N_2 fixation. A significantly positive relation was found for soil N uptake and shoot N content in both soils, indicating mungbean relied less on biological N_2 fixation for its N requirements than on soil N uptake (Fig. 4). Rao and Ali (2006) also reported that mungbean genotypes in the presence of more soil N enhanced amounts of N in plant biomass.

Conclusion

Estimation of N_2 fixation by mungbean in two soil textures using the ^{15}N the natural abundance technique revealed that plant growth and symbiotic fixation of N differed between genotypes. Plant growth parameters, N_2 fixation, and soil N uptake had significantly higher responses in the silt loam soil compared to loamy sand. These results indicate the role of soil properties in affecting plant development and subsequent N_2

fixation. Among genotypes, IC 8972-1 recorded the highest dry matter yield, shoot N content, and soil N-uptake, indicating that genetic constitution could also influence plant growth and performance of N₂ fixation in mungbean. We found that mungbean derived up to 42.9% of its N from the atmosphere and contributed up to 80.0 mg plant⁻¹. Significant correlations between dry matter and N-fixed, δ¹⁵N (‰) and soil N uptake, %Ndfa and soil N uptake, shoot N content and soil N uptake indicate plant growth and soil properties could alter N₂ fixation. Thus, results from this study demonstrate that mungbean can contribute to N additions in agricultural systems if proper genotypes are grown in suitable soil conditions.

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