Explore the Utilization and Nutrition of Mungbean [*Vigna Radiata* (L.) R. Wilczek] for Human Consumption to Promote in Senegal and Virginia

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ABSTRACT

With a rapidly growing world population and increased threats of climate change, Sub-Saharan Africa is most at risk for lower crop yields and facing hunger. Within Sub-Saharan Africa, Senegal has some of the highest levels of anemia and micronutrient deficiencies among women and children. Mungbean [*Vigna radiata* (L.) R. Wilczek] is a pulse crop that has recently been successfully introduced in Senegal to diversify a primarily cereal-based diet consisting of millet, maize, and rice. The potential for mungbean to be harvested as a leafy green as well as a pulse could allow for a more balanced and nutrient-rich diet. This diversification could help combat micronutrient deficiencies while earlier harvest of the leaves could help alleviate the pressures of the "hunger season." To understand the effects of leaf harvest on mungbean grain yield, yield components, and nutrition, a field trial was conducted in Blacksburg, Virginia for three consecutive years from 2020 to 2022. In a split-plot design, four frequencies of leaf harvest (0x, 1x, 2x, and 3x) on seven accessions of mungbean were tested in triplicate. The objective of this experiment was to determine if mungbean can be used as a dual-purpose crop as a leafy green vegetable without decreasing grain yield in Senegal. It was found that mungbeans could undergo up to two leaf harvest of immature leaves without reducing yield, total dry matter (TDM), or yield components. The harvested leaves were also found to be highly nutritious with 22.0% protein, 12.3% fiber and 8.5% ash with no significant differences between leaf harvest treatments. These results indicate that mungbean can be used as a dual-purpose crop for harvest as leafy greens and pulse in Senegal.

Further, mungbeans were studied as a viable crop in southside Virginia. The objective was to evaluate the efficacy of mungbeans as an alternative crop to tobacco farmers in Virginia. Field trials were conducted on farmers' fields and at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center in 2021 and 2022. A split-plot experimental design was used with early and late planting dates in the beginning and end of June as the whole plots and two commercially-available cultivars, Berken and OK 2000, as the sub-plots. Due to highly variable rain patterns in 2021 and 2022, as well as differing management practices, there were no consistent effects of genotype or planting date on yield, plant height, pods per plant, seeds per pod, or seed size. Yield ranged from 0.19 MT ha^{-1} to 1.18 MT ha^{-1} with an average yield of 0.84 MT ha^{-1} in 2021 and 0.38 MT ha^{-1} in 2022. Though there was variation in yield, across planting dates, cultivars, locations, and years, the highest yield was higher than global averages. It was concluded that while there is great potential with the growing mungbean market, more studies of breeding and supply chain issues and development of a production guide are needed for mungbean to be successful in Virginia. A final study compared soybean, edamame, and mungbean nutritional components and volatiles, two characteristics of importance to breeding objectives and food processing regarding plant alternative proteins. It was found that mungbean had significantly less protein (21.1%) than soy (36.2%) and edamame (38.3%). Mungbean also had lower fat (0.769%) compared to soy (13.5%) and edamame (14.0%). Analysis of aromatic compounds revealed that soybean, edamame, and mungbean each had unique profiles that could be advantageous to the production of specific plant protein foods. Overall, these studies demonstrate the growing importance and potential of mungbean in both Senegal and in the United States.

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GENERAL AUDIENCE ABSTRACT

With a rapidly growing world population and increased threats of climate change, Sub-Saharan Africa is most at risk for lower crop yields and facing hunger. Within Sub-Saharan Africa, Senegal has some of the highest levels of anemia and micronutrient deficiencies among women and children. Mungbean [*Vigna radiata* (L.) R. Wilczek] is a pulse crop that has recently been successfully introduced in Senegal to diversify a primarily cereal-based diet consisting of millet, maize, and rice. The potential for mungbean to be harvested as a leafy green as well as a pulse could allow for a more balanced and nutrient-rich diet. This diversification could help combat micronutrient deficiencies while earlier harvest of the leaves could help alleviate the pressures of the "hunger season." To understand the effects of leaf harvest on mungbean grain yield, yield components, and nutrition, a field trial was conducted in Blacksburg, Virginia for three consecutive years from 2020 to 2022. In a split-plot design, four frequencies of leaf harvest (0x, 1x, 2x, and 3x) on seven accessions of mungbean were tested in triplicate. The objective of this experiment was to determine if mungbean can be used as a dual-purpose crop as a leafy green vegetable without decreasing grain yield in Senegal. It was found that mungbeans could undergo up to two leaf harvest of immature leaves without reducing yield, total dry matter (TDM), or yield components. The harvested leaves were also found to be highly nutritious with 22.0% protein, 12.3% fiber and 8.5% ash with no significant differences between leaf harvest treatments. These results indicate that mungbean can be used as a dual-purpose crop for harvest as leafy greens and pulse in Senegal.

Further, mungbeans were studied as a viable crop in southside Virginia. The objective was to evaluate the efficacy of mungbeans as an alternative crop to tobacco farmers in Virginia. Field trials were conducted on farmers' fields and at Virginia Tech's Southern Piedmont Agricultural Research and Extension Center in 2021 and 2022. A split-plot experimental design was used with early and late planting dates in the beginning and end of June as the whole plots and two commercially-available cultivars, Berken and OK 2000, as the sub-plots. Due to highly variable rain patterns in 2021 and 2022, as well as differing management practices, there were no consistent effects of genotype or planting date on yield, plant height, pods per plant, seeds per pod, or seed size. Yield ranged from 0.19 MT ha^{-1} to 1.18 MT ha^{-1} with an average yield of 0.84 MT ha^{-1} in 2021 and 0.38 MT ha^{-1} in 2022. Though there was variation in yield, across planting dates, cultivars, locations, and years, the highest yield was higher than global averages. It was concluded that while there is great potential with the growing mungbean market, more studies of breeding and supply chain issues and development of a production guide are needed for mungbean to be successful in Virginia. A final study compared soybean, edamame, and mungbean nutritional components and volatiles, two characteristics of importance to breeding objectives and food processing regarding plant alternative proteins. It was found that mungbean had significantly less protein (21.1%) than soy (36.2%) and edamame (38.3%). Mungbean also had lower fat (0.769%) compared to soy (13.5%) and edamame (14.0%). Analysis of aromatic compounds revealed that soybean, edamame, and mungbean each had unique profiles that could be advantageous to the production of specific plant protein foods. Overall, these studies demonstrate the growing importance and potential of mungbean in both Senegal and in the United States.

DEDICATION

To my niece, Elizalee, and my nephews, Ethan and Elliott,

May you always pursue learning.

May your passions shape you but your faith lead you.

I dedicate this work to you.

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LIST OF ACRONYMS

CLS	Cescospora leaf spot
DDS	Dietary Diversity Score
GC-MS	Gas chromatography-mass spectrometry
GHG	Greenhouse gases
GDD	Growing Degree Days
LHF	Leaf Harvest Frequency
MT	Metric ton
MYMD	Mungbean yellow mosaic disease
NDF	Neutral detergent fiber
NPK	Nitrogen, phosphorus, potassium
SPAREC	Southern Piedmont Agricultural Research and Extension Center
TVP	Texturized vegetable protein
TDM	Total dry matter
TIU	Trypsin inhibitor units
USD	United States Dollar

Chapter 1. Introduction

Context and Justification

The world population is currently growing by 83 million people annually with a trajectory of 9.8 billion by 2050 and 11.2 billion by 2100 (United Nations, 2022). To sustain this population with current production and consumption practices, 25-70% more food will need to be produced (Hunter et al., 2017). Meanwhile, 811 million people in the world face hunger and one out of every five people face hunger in Africa (FAO et al., 2021). Even if food production increased enough to feed the world population, the carbon footprint and release of greenhouse gases (GHGs)would also need to be reduced as climate change becomes an increasing concern.

Sub-Saharan Africa is most at risk from natural disasters, flooding, or drought resulting from climate change (Thompson et al., 2010). In addition to hunger-stricken diseases such as wasting (low weight-for-height) and stunting (low height-for-age) which have visible symptoms, much of Sub-Saharan Africa suffer from hidden hunger, or micronutrient deficiencies which may have no visible symptoms (FAO, 2018). Diversifying crops is therefore important to manage these risks and to increase dietary diversity to ensure proper nutrients as well as proper caloric intake.

In Senegal, located at the furthest western point in Africa, nearly half of deaths in children under five are related to malnutrition (UNICEF, 2021). Stunting affects 15% of children under five and wasting affects 5.9% of children (UNICEF et al., 2022) while 71% of children are anemic (USAID, 2021). The Senegalese diet primarily consists of cereal grains such as millet, maize, and rice which lacks the essential amino acid, lysine, and limits the diversity of minerals and vitamins (Hou et al., 2019). Introducing legume crops and leafy green vegetables in addition to cereal crops can create a balanced diet with all nine essential amino acids and high mineral and vitamin content (Hou et al., 2019). Though groundnut and cowpea are commonly grown in

Senegal, more legume and leafy green vegetable crops are urgently needed to diversify diet and improve micronutrient deficiencies in Senegal.

A legume crop that has already found a lot of recent success in Senegal is mungbean. Mungbean [*Vigna radiata* (L.) Wilczek] is a pulse crop in the Fabaceae family that is native to India. Mungbean is also in the same genus as cowpea [*Vigna unguiculata* (L.) Walp.], a native legume crop in Senegal. Mungbean's similarity to cowpea has allowed the crop to be more easily and culturally accepted by farmers due to similar production and utilization practices. In addition, mungbean is rich in protein, amino acids, dietary fiber, iron and other minerals, and vitamins. Because of its highly nutritious composition, mungbean is proven to have human health benefits such as the reduced risk of hyperglycemia, hyperlipemia, hypertension, cancer and melanogenesis (Hou et al., 2019). Mungbeans are also greatly drought and heat tolerant with a short growing season of only 60-80 days (Myers, 2003). The short growing period and earlier harvest can shorten the hunger season, or the period between planting and harvest when food supply is limited. Like other legumes, mungbean fixes nitrogen in the soil through the formation of rhizobium nodules on their roots and can improve overall soil health.

Virginia Tech has successfully introduced mungbean to the crop production and diets of twenty-five villages in Senegal. Research projects through Virginia Tech have been conducted on mungbean in the intercropping system (Diatta et al., 2020b; Trail et al., 2016), on the overall cultural acceptance of mungbean and improved dietary diversity (Vashro, 2017), on the effects of inoculating mungbean with nitrogen-fixing bacteria, *Bradyrhizobium* (Diatta et al., 2020a; Mott, 2022) and on soil texture in mungbean production (Diatta et al., 2020a). Mungbean as a pulse crop has many benefits, but there is further potential for mungbean leaves to also be consumed. Native cowpea leaves are traditionally consumed in Eastern African countries such as

Kenya and Ethiopia as well as specific villages in Senegal as leafy greens (Owade et al., 2021). The ability for mungbean to be harvested as a leafy green can potentially add a second food source with diverse nutrients as well as provide an earlier harvested crop.

In addition to studying the utility of mungbean as a leafy green crop in Senegal, we are exploring the potential of mungbean as an alternative crop to tobacco for Virginia farmers. Tobacco production has reduced from 53,770 acres in 1997 to 23,039 acres in Virginia in 2017 (USDA, 2017). Meanwhile, plant alternative proteins are growing substantially in demand in the United States. Mungbean is already being used in products such as Beyond Burger, a plant-based meat patty (Beyond Beef), and JUST Egg, a plant-based egg substitute (JUST Egg). Growing demand for plant-based proteins and the existing market for mungbeans in Asian cuisine has created a 111% increase in mungbean imports to the United States from the 2018/2019 market year to the 2020/2021 market year (USDA, 2022). The short growth cycle of mungbean could allow for double-cropping with winter wheat without reducing pulse yields. The ability of mungbean to fit into common mid-Atlantic crop rotations could provide Virginia tobacco farmers with a new, highly profitable vegetable crop in a growing market.

Lastly, as the alternative protein market grows, we wanted to explore nutritional aspects and volatile compounds in three important plant proteins, soybean, edamame, and mungbean. The purpose is for the results to be used for future food processing and even breeding selections.

The overall goals of the three studies are to explore the feasibility to utilize mungbean as a dual-purpose crop in Senegal and an alternative crop in Virginia based on its agronomic traits and seed composition profiles. The specific objectives are 1) to investigate the effects of various levels of manual leaf harvest (as a leafy green for human consumption) on mungbean grain yield,

yield components (plant height, biomass, pods per plant, seeds per pod, and seed size), and leaf nutrition ; 2) to exam the feasibility of mungbean production in Southside Virginia; and 3) to compare the nutritional and volatile profiles among mungbean, edamame (vegetable soybean) and soybean.

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Chapter 2. Literature Review

Adaptation

Mungbean is a very important legume in Asian countries, originating in India and Thailand (Lambrides & Godwin, 2007). It is well known for its high nutrient content, short growing season, drought tolerance, and health benefits. Mungbean can be used for human consumption as a pulse or bean sprout and can also be grown as a forage or cover crop.

Mungbean is highly drought tolerant and can thrive in less than two-thirds the amount of water compared to soybean (TABLE 2-1). It is a warm-season crop adapted to tropical and subtropical climates (Lambrides & Godwin, 2007). Mungbeans do best on fertile, sandy loam soils with good internal drainage and a pH in the range of 6.3 and 7.2. (Lambrides & Godwin, 2007). When calculating Growing Degree Days (GDD), 7.9 ^{o}C is the base (Ellis et al., 1994) with 1,150.5-1,430.5 GDD from planting to harvest (Zargaran Khouzani, 2021). Most varieties have a growing season of 70-90 days (Lambrides & Godwin, 2007), however, some shorter-cycle varieties have been developed to mature in only 55-65 days (Chadha, 2010). The short growth cycle gives the possibility for multiple successive plantings and harvest within one season. In addition, many genotypes have indeterminant growth habit which allows for multiple harvests of pods from each plant which is desirable for subsistence farming. There are also some determinant genotypes with pods that mature simultaneously which are more desirable for mechanized harvest in large-scale agriculture.

TABLE 2-1 Environmental factors, growth cycle and added market values between soybean and mungbean.

	Soybean	Mungbean
Temperature	21-32ºC ("Glycine max (soyabean)," 2019)	25- 35ºC (AVRDC The World Vegetable Center, 2016)
Water requirements	600-800 mm / season (Harris & Mace, 2012)	400-550 mm / season (AVRDC The World Vegetable Center, 2016)
Heat/drought tolerant	Drought stress at flowering reduces yield, significantly decreases yield at pod-filling stage ("Glycine max (soyabean)," 2019)	Highly drought and heat tolerant (Myers, 2003)
Growth cycle (number of days from planting to harvest	70 to 160 days ("Glycine max (soyabean)," 2019)	60-80 days (Myers, 2003)
Price/pound	$16.10/ bushel or $0.27/ lb (May 2022) (USDA, 2022a)	$0.73/ lb (May 2022) (USDA, 2022b)
Sprout	Can be used for sprouts but not common.	The majority of bean sprouts are produced from mungbean

Morphology

Like other legumes, mungbean can fix nitrogen due to a symbiotic relationship with *Rhizobium* through nodules in the roots. Mungbeans can have prostrate, suberect, or erect growth habits ranging from 0.15 to 1.25 m tall (Lawn & Russell, 1978). The leaves are pinnate trifoliate with a larger terminal leaflet perpendicular to two smaller primary leaflets (Lambrides & Godwin, 2007). The leaves, stems, pods, and roots are pubescent. The flowers are hermaphroditic and the petals are yellow or pale yellow. The style is curved and encapsulated with 10 anthers by the keel. There are two winged petals protected by a two-lobed standard petal and the buds are curved, similar to the head of a scythe (Lambrides & Godwin, 2007).

The seed size of mungbean ranges from 1.58 to 7.89 g/100 seeds (Gayacharan et al., 2020). The small seed allows for a higher seed-to-pod ratio varying from five to seventeen seeds per pod, depending on the genotype (Gayacharan et al., 2020). Seed coat colors may vary by black, green, brown, or yellow, explaining the alternative common names for mungbean: green gram, golden gram, and black gram (Lambrides & Godwin, 2007). Despite differences in external color, all mungbean seeds have yellow endosperm. The seed coats may also vary in luster with some appearing glossy, while others are more dull and matte in appearance (Lawn & Russell, 1978). Mungbean pods are long and slender, ranging from 12-16 cm long (Gayacharan et al., 2020). The pods cluster around a leaf axil and each plant typically has 30 to 40 pods (Myers, 2003). When fully mature, the pods turn completely black and lose flexibility. The leaves senesce and drop when the last pods begin to mature (Ellis et al., 1994).

Cultivation

Row spacing

It is widely accepted that mungbean performs better with closer row spacing due to shading out competitive weeds than that of larger rows of 50 cm or more. In India, interrow spacing of 43 cm and intrarow spacing of 7 cm gave a maximum height at 47.50 cm (Ihsanullah, 2002). Other yield components such as pods per plant, the number of seeds per pod, biomass, yield, and seed weight were all maximized by having interrow spacing of 20 cm and intrarow spacing of 15 cm (Ihsanullah, 2002).

In Australia, Rachaputi et al. (2015) found that in 30 cm row spacing, the fraction of absorbed sunlight was above 0.9, while in 100 cm row spacing, it was less than 0.7. This difference in row spacing resulted in an 11% increase in leaf area development for 30 cm rows and increased the overall yield for narrow rows (Rachaputi et al., 2015).

Harvest and Storage

In smallholder farming, mungbean is almost entirely harvested by hand. This gives the benefits of having multiple harvests due to the indeterminate growth pattern. In mechanical harvesting, a combine is used and only one harvest can be completed. Typically, mechanical harvest is completed when 75% of the pods are mature but decreases overall yield by not having multiple harvests. In Myanmar, smallholder farmers expressed the need for mechanization because they can harvest quickly in the case of a large storm that would otherwise wash out their yield (Depenbusch et al., 2021). In large-scale mungbean farming, a desiccant must be sprayed to ensure all of the leaves senesce and stems dry out before combining (Padmaja et al., 2013). In colder climates, timing with the first frost may also be enough for the leaves to senesce.

Mungbean should be stored in a cool, dry place out of the sun. Ideal seed storage is in a silo with a temperature of 10-20 ˚C. Proper storage will help with germination vigor as well as protect the seed from pest buildup (Australian Mungbean Association, 2017). Seed storage moisture should be around 12%. Any moisture substantially higher can increase respiratory activity, reducing seed energy reserves and limiting seed vigor (Ziegler et al., 2020).

Fertilization

Like other legume crops, mungbean can fix nitrogen when in a symbiotic relationship with rhizobium, soil-dwelling bacteria that form root nodules on legume. Rhizobium may be residual in the soil or may need to be inoculated on the seed for nitrogen fixation to occur. In China, Yin et al. (2018) found that applying additional nitrogen to mungbeans can actually improve yield, number of pods per plant and seed size to help the plant in early stages when nodules are still forming, but the application rate peaked at 47.20 Kg ha^{-1} N before yield began to decline. In Bangladesh, a similar result showed that applying 60 Kg ha^{-1} N gave the optimal yield, but higher nitrogen input would begin to decrease yields (Razzaque et al., 2017). A study in Pakistan compared inorganic NPK inputs to organic fertilizers such as poultry litter, farmyard manure, and biofertilizer. The NPK fertilizer resulted in the highest yield and yield components, but poultry litter was second best and deemed the have the most economical return out of any of the fertilizers (Naeem et al., 2006).

Potassium and phosphorous fertilizer are also considered to be important in improving mungbean production. Applying potash at 90 Kg ha^{-1} resulted in maximum plant height, pods per plant and yield in Pakistan (F. Hussain et al., 2011). Phosphorus applied at 40 Kg ha-1 in combination with 1 Kg ha-1 molybdenum and rhizobium resulted in the highest uptake of nitrogen in the plant shoot, optimizing plant yield and yield components (Rahman et al., 2008). In addition to molybdenum, a 3-year study found that other micronutrients sulfur, boron, and zinc can increase mungbean yields by 24-54% when applied with nitrogen and phosphorus (Sahrawat et al., 2010).

Disease, insect and weed control

Biotic Factors

Mungbean yellow mosaic disease (MYMD) is an important viral disease of mungbean, transmitted by whitefly, but MYMD-resistant varieties are being developed (Nair et al., 2019). Yield can be greatly compromised by MYMD and has led to yield decreases in Pakistan of 32.2-78.6% and is the primary reason for low yields in India (Khattak et al., 2000). Anthracnose, powdery mildew, Cescospora leaf spot (CLS), dry root rot, halo blight, and tan spot are all fungal diseases of mungbean that cause significant economic damage (Nair et al., 2019). Stem- and pod-borers along with jassid and white fly are insect pests of mungbean (Sehrawat et al., 2015). Many of the yield-limiting biotic factors can be controlled through breeding technology and better cultivars. Also, these diseases and pests are common in south Asia but pose less threat to the United States and Sub-Saharan Africa. Weed pressure can also significantly reduce yield since mungbean are not very competitive seedlings. Weeds can mostly be controlled through row spacing and herbicide control if necessary (Nair et al., 2019).

Abiotic Factors

Mungbean is a drought-tolerant crop and is averse to waterlogging and frost (Cotton Research and Development Corporation, 2012; Nair et al., 2019) Also, soil with high salinity can limit root water uptake and absorption of necessary nutrients (Mohammed, 2007). Salt stress can decrease seed germination as well as seedling growth rate (Cotton Research and Development Corporation, 2012). High soil salinity is most likely to occur during rainy seasons or in waterlogged soil because of the lack of proper drainage. Instead of washing away salts through water drainage, waterlogged soils accumulate high concentrations of salt as the water evaporates (Dikeogu et al., 2021). In later development stages, high salinity in mungbeans can force early desiccation, flower abortion, and pod shattering (Sehrawat et al., 2015).

Defoliation Effects

Defoliation of mungbean leaves from either the top 25% or basal 25% leaves was not found to decrease seed yield or total dry matter (TDM) (Monjurul Mondal et al., 2011). In the same study, it was found that removing the top 25% - 75% of leaves increased number of branches with the more leaves defoliated, the more branches grown (Mohd Mondal et al., 2011). Similarly in cowpea, 100% defoliation of the plant resulted in the highest number of new leaves (35 leaves higher than the control) (Biswas et al., 2004). It was also found that cowpea could be defoliated up to 33% without effecting yield or TDM (Biswas et al., 2004).

Source-Sink Relationship

The source-sink relationship in plants indicates what part of the plant gains assimilates from photosynthesis, known as the source, and what part of the plant uses the assimilates from photosynthesis to grow, known as the sink (Venkateswarlu & Visperas, 1987). Roots, young leaves, flowers, and fruit are all common plant sinks whereas developed leaves are the most common source of assimilates for plants (World Health Organization, 2022). For example, when deflowered, it was found that a mungbean plant had a higher photosynthetic rate and the leaves had a higher concentration of nitrogen than the control plant. It was then found that leaves, stem, roots and nodules of the plant had increased from 62.2 to 142.4% in dry weight (Mitra & Ghildiyal, 1988). This is likely due to the fact that the flower is a sink for the plant and upon removal, the plant reallocates its resources to an “alternative sink” (Mitra & Ghildiyal, 1988). The same study evaluated the effect of removing all but one of the source leaves on the photosynthetic rate. Within 24 hours of the non-source leaves, the remaining leaf increased in photosynthetic rate by 28.5%. (Mitra & Ghildiyal, 1988). A further study on mungbean source-sink relationships showed that depodding a plant increased overall photosynthetic rates (Chen & Sung, 1982).

Mungbean for Human Consumption, Forage, and Cover Crop

Since mungbean is consumed around the world, there are a variety of uses, forms, and dishes. In south Asia (India, Pakistan, Bangladesh, Nepal), mungbean is typically made into dahl. Dahl refers to dried split pea, bean, or lentil, and as a way of cooking that boils down the bean into a highly spiced stew-like dish. It can also be fermented or milled into flour (Lambrides & Godwin, 2007).

In eastern and southeastern Asia, mungbean is often used to make bean sprouts. In Korea, the sprouts are blanched in a dish called sukju namul. The sprouts are considered a higher price commodity as they have a shelf life of only seven days and must be eaten fresh. Mungbean sprouts are preferred with short, crisp roots, and small cotyledons (Lambrides & Godwin, 2007). In the US, mungbeans are mainly consumed as bean sprouts in salads or in Asian cuisine (Fery, 2002). In Indonesian cuisine, sprouts are incorporated in many recipes including: tauge, a tofu stir-fry; gado gado, a salad with peanut sauce; soto ayam, a chicken soup; and orak-arik, a vegetable and egg stir-fry (Lambrides & Godwin, 2007).

In China, mungbean can be cooked with rice and sugar as a sweet dessert (Lambrides & Godwin, 2007). Mungbean is also used in the Chinese noodle, vermicelli, sometimes referred to as glass noodles (Chauhan & Williams, 2018). In Korean cuisine, mungbean can be ground for stuffed pancakes called binaetteok (Lambrides & Godwin, 2007).

Mungbean can also be used as forage as it is palatable to livestock and highly nutritious (Alsheikh, 2019). Because mungbean has fewer sulfur-based amino acids and has more easily digestible starches than other legumes, flatulence and bloating is not a concern (Sandhu & Lim, 2008). It has a forage-feeding value 75-80% that of alfalfa hay (Boe et al., 1991), and is suitable for fodder, silage, or grazing (Alsheikh, 2019).

Mungbean is sometimes used as a cover crop due to its short growing season and its ability to add nitrogen into the soil. Mungbean can add 2.9 tons of biomass per acre to the soil and its biomass is comprised of 3.92% nitrogen and 40.4% carbon. Out of nine other major legume cover crops including Alfalfa and cowpea, mungbean has the highest percent nitrogen contribution to soil (Hoorman et al., 2009). Mungbeans are grown in rotation with rice and rice-wheat systems in India as a cover crop that adds nitrogen back into the soil (Nair & Schreinemachers, 2020).

Nutritional Factors & Health Benefits

Mungbeans are high in flavonoids, antioxidants, polyphenols, polysaccharides, and peptides which have anti-inflammatory, cardio-protective properties, and are associated with the prevention of cancer, hyperglycemia, hyperlipemia, and hypertension (Chitra et al., 1995; Hou et al., 2019; Ullah et al., 2020). Because of this, mungbeans have been used throughout history in Chinese traditional medicine (Hou et al., 2019).

In addition to bioactive compounds, mungbeans are high in protein, fiber, phosphorous, and potassium as well as essential micronutrients such as folate, iron, magnesium, and zinc. When comparing one cup of raw cowpea, millet, unenriched white rice, and mungbean, mungbean is the highest in protein (49.4 g), iron (14.0 mg), magnesium (391 mg), phosphorus (2579.0 mg), potassium (2579.0 mg), folate (1294.0 mcg), and fiber (33.7 g) (USDA, 2019). Folate, iron, magnesium, and zinc are critical for development and even more vital for children under five. Deficiencies in these essential micronutrients can lead to birth defects, low birth weights, cognitive development issues, and weaken immune systems (Sandberg, 2002; Singh, 2018). These micronutrients have been fortified in cereal-based foods in the United States since the 1940s when flour was first "enriched" with iron. Now cereal and flour are commonly fortified with magnesium, folate, and zinc in addition to iron in the United States (Sandberg, 2002). In many developing countries, foods are not typically fortified and in cereal-based diets alone, there are not sufficient nutrients which can result in anemia and "hidden hunger." Hidden hunger is the "presence of multiple micronutrient deficiencies in the absence of an energy-deficit diet (Lowe, 2021)," or in other words, a person may intake enough calories, but not micronutrients for proper development. Legumes, like mungbeans, have the ability to provide these nutrients in a natural diet.

It is important to recognize that, like other legumes, mungbeans have antinutritional factors such as phytic acid which can lower the availability of necessary minerals such as zinc, iron, and magnesium. However, mungbeans have a lower phytic acid content (12.0 mg/g) compared to soybean (36.4 mg/g), urd bean (13.7 mg/g), and pigeonpea (12.7 mg/g) (Chitra et al., 1995). Phytic acid is also significantly reduced by different food preparation methods. Cooking breaks down phytic acids at high temperatures (Barakoti & Bains, 2007) and soaking for 12 hours reduced phytic acid by 13-41%, while soaking for 12 hours and germinating for 48 hours reduced phytic acid by 60-73% (Tajoddin et al., 2011). During germination, for consumption as sprout, ascorbic acid also increases which helps with iron uptake (Barakoti & Bains, 2007). Trypsin inhibitor is another antinutritional factor that disrupts trypsin, a digestive enzyme that helps absorb nutrients. High levels of trypsin inhibitor units (TIU) can be found in soybean, but mungbean has nearly 50% less TIU than soybean (Savage & Morrison, 2003). Guillamón et al. (2008) found that soybean ranged from 43-84 TIU, while Dahiya et al. (2015) found that mungbean only had 17.3 TIU. Though trypsin inhibitor is low in mungbean, it can also be significantly reduced during cooking processes.

Mungbeans are also rich in most of the essential amino acids, including lysine, but are limited by the sulfur-containing amino acids, methionine and cysteine (Akpapunam, 1996). On the other hand, cereal crops are high in sulfur-containing amino acids, but low in lysine (Hou et al., 2019). Proteins that contain all nine of the essential amino acids such as animal products are considered “complete proteins” (Bohrer, 2019). While pulses are not complete proteins on their own, when combined with cereal grains all essential amino acids are present which makes cereal and legume crops complementary proteins (Hertzler et al., 2020). The need for both legumes and cereal grains for complete protein emphasizes the need for a balanced diet with diverse food choices.

Global Market

Globally, 5.3 million tons of mungbeans are produced were ~7.3 million Ha in 2019 (Pratap et al., 2020). The top producing countries are India and Myanmar, each with about 30% of global production, and China with about 16% of global production (World Vegetable Center, 2019). Yield varies from 0.5 to 1.5 MT ha^{-1} while the global average is 1.2 MT ha^{-1}. Despite India being a main producer at 1.6 million to 2.17 million MT of mungbeans annually, it is sown on 3.8 million to 4.32 million hectares for an average yield of only 0.50 MT ha^{-1} (Indian Institute of Pulses Research, 2018; Mishra et al, 2022; Nair & Schreinemachers, 2020). The production of mungbean in Myanmar has grown significantly from 0.04 million hectares in 1980 to 0.74 million hectares in 2000 to 1.21 million hectares in 2016 after extensive breeding efforts (Central Statistical Organization, 2000-2017; Sequeros et al., 2020). Like India, Myanmar also produces 1.6 million tons of mungbeans annually (Nair & Schreinemachers, 2020), but has much higher yields at 1.3 MT ha^{-1} (Sequeros et al., 2020). Approximately 92% of Myanmar's mungbeans are exported (Nair & Schreinemachers, 2020) which provides 70% of India's mungbean imports (Pratap et al., 2020). China produced 690,000 tons of mungbean in 2014. The yield efficiency is much higher than that of India at 1.41 MT ha^{-1} (Li et al., 2017; Ministry of Agriculture of China, 2015). Most of the production in China is consumed domestically but 140,000 tons were still exported in 2008 (Chauhan & Williams, 2018).

Southeast Asian countries including Indonesia, Vietnam, and Thailand are large mungbean producers with productions of 244,000 MT, 92,000 MT, and 86,000 MT, respectively from 2016-2017 (Sequeros et al., 2021). In these countries, mungbean is primarily produced by smallholder farmers which struggle with high production costs due to the lack of mechanized planting and harvest (Chauhan & Williams, 2018).

Australia is a rapidly growing producer of mungbean as well with production growing from about 50,000 tons in 2005 to 143,000 MT in 2016. Although production land area was increased, a lot of the increase in Australia's mungbean production was due to improvements in technologies, practices, and cultivars (Chauhan & Williams, 2018). About 90% of Australia's mungbeans are exported to countries including India, Indonesia, Vietnam, Sri Lanka, and Thailand (Chauhan & Williams, 2018).

Mungbean is also grown in Africa, mainly in Kenya, Tanzania, and Uganda with 149,000 MT, 73,000 MT, and 33,000 MT grown, respectively, from 2016-2017. Kenya is by far the largest mungbean consumer and importer in Africa (Sequeros et al., 2021).

The United States production of mungbean is very limited with only 100,000 acres, almost entirely located in Oklahoma. Nearly 75% of mungbeans consumed in the US are imported. Imports of mungbean are trending upwards with 12,731 MT, 13,474 MT, and 13,672 MT imported in 2012, 2013, and 2014, respectively. Imports were worth approximately 22 million USD in 2014 and are continuing to grow (Bhardwaj & Hamama, 2015).

Economic Potential

As the demand grows and technologies develop for meat alternatives, the market for texturized vegetable protein (TVP) is expected to increase to 21.23 billion USD by 2025 (Zion Market Research, 2019). Mungbean protein isolate is suggested as a plant-based ingredient in these meat analogues (Brishti et al., 2020; Schlangen et al., 2022). In the first half of 2021, mungbean imports to the United States increased by 62% compared to 2020 (Batzer et al., 2022; NASS, 2021). Even in non-vegetarian products, mungbean is a proposed binder in food processing. In a study by Modi et al. (2004), soybean, bengal gram and mungbean were evaluated for use as a binder in a buffalo burger. In this study, mungbean had the highest yield at 95.7%, lowest shrinkage at 5% and lowest fat absorption when frying and best sensory analysis.

In addition to mungbean's potential as an alternative plant protein, it also continues to be in demand for Asian cuisine, and yields will likely need to improve to meet demands. Consumption of mungbeans has increased from 22-66% in various countries (Shanmugasundaram et al., 2009). Breeding programs have found great success and economic return by improving yields through disease resistance and by creating determinant, erect varieties that are easier to mechanically harvest. Currently, mungbean is Myanmar's second most produced crop, next to rice, and is primarily produced by smallholder farmers. In 1981, Myanmar invested 5 million USD into breeding research and the adoption of new cultivars. Myanmar has received an estimated return on investment of 92 USD in return for every dollar spent (Sequeros et al., 2020). As of 2016, the benefits totaled 1.4 billion USD and 95% of this return has gone to smallholder farmers. It is estimated that by 2030, Myanmar will have a return of 3.7 billion USD (Sequeros et al., 2020).

With its growing demand and increasing yields, mungbeans have the potential to be an economically, environmentally, and nutritionally important crop in both the United States and in Senegal.

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Chapter 3. The Influence of Leaf Harvest on Growth, Biomass, Grain Yield, and Nutrient Value of Mungbean [*Vigna radiata*] for Human Consumption

The Influence of Leaf Harvest on Growth, Biomass, Grain Yield, and Nutrient Value of Mungbean for Human Consumption

Jessica Wilbur

Abstract

Mungbean [*Vigna radiata* (L.) R. Wilczek] has been successfully introduced as a pulse crop in Senegal in recent years due to its short growing season, drought tolerance, and similarities to native cowpea. Since the traditional Senegalese diet is mostly comprised of cereal grains, there is little dietary diversity which has resulted in an extremely high prevalence of anemia and micronutrient deficiency. The consumption of mungbean leaves as a vegetable green has the potential to diversify micronutrient intake as well as harvest an earlier crop and shorten the time between planting and harvest when food sources are scarce also known as the hunger season. The objective of this project is to determine if mungbean can be used as a dual-purpose crop, harvested as both a leafy green and as a grain legume, for human consumption without compromising grain yield, seed quality, and nutritive value. The experiment was conducted in Blacksburg, VA for three consecutive years from 2020 to 2022 in a split-plot design. There were four frequencies of leaf harvest (0x, 1x, 2x, 3x) as the whole plots and seven different accessions as the sub-plot with three replications each. Harvested leaves were defined as young, immature leaves that were fully expanded and connected to branches, not the main stem. Leaves were harvested in accordance with the leaf harvest frequency treatments beginning at week seven post-planting and repeated every successive week until the third leaf harvest in week 10. Leaf and grain yield data was collected as well as yield components, pods 10 plants^{-1}, seeds pod^{-1}, seed size, plant height, and plant dry matter. A mixed-model ANOVA was used to interpret the data. The results revealed that there was no significant difference between overall grain yield or yield components when leaves were harvested up to two times, but that the accessions did have a

significant impact on overall grain yield. This suggests there is potential for using mungbean as a dual-purpose crop without significantly decreasing bean yield and adding another source of food to Senegalese diets through the consumption of the leaves.

Introduction

In Senegal, malnutrition is high with 18% of children under five facing stunting, 14% of children underweight, and 8% of children wasting. Micronutrient deficiencies, or "hidden hunger" are even drastically higher with 54% of women of reproductive age and 71% of children suffering from anemia (USAID, 2021). A large issue in nutrition in Senegal is due to the hunger season. Hunger season is the period of time after planting and before harvest when food supplies are getting lower. In Senegal, the hunger season is during the rainy season from June to September (USAID, 2021). A crop with a shorter growing season can help alleviate some of the burden of this period.

Mungbean [*Vigna Radiata*] is a short-season pulse crop that can mature in 60-90 days, depending on the variety (Myers, 2003). Because it is a legume, it has many benefits for human health including high protein, minerals, and vitamins. These all contribute to lower risk of hypertension, diabetes, heart disease, and cancer (Hou et al., 2019). In addition, mungbean can fix nitrogen which can improve overall soil health (Lawn & Russell, 1978).

The introduction of mungbean has already been successful in multiple villages in Senegal. Women of these villages have had a positive response towards mungbean saying that mungbean keeps them full for longer and it is easier to harvest than native cowpea because the pods cluster around one central axis (Vashro, 2017). Despite overall approval of mungbean by women interviewed, the dietary diversity score, was not any higher from women that ate mungbean than women that did not. Dietary diversity score (DDS) refers to a “qualitative measurement of food consumption that reflects household access to a variety of foods” defined by the FAO (2021) and is an indicator of a balanced, nutritious diet. In order to improve DDS in Senegal, food groups including dark leafy greens, legumes and pulses, and nuts and seeds can be added (Vashro, 2017).

This study explores the efficacy of mungbean to be used as a dual-purpose crop in the form of a leafy green vegetable and as a bean crop. It is important that harvesting for leaves does not negatively affect overall grain yield. If mungbean is utilized as two vegetable crops, it can significantly shorten the hunger season, provide more diverse nutrients in the Senegalese diet, and provide higher crop diversity to manage environmental risks.

Materials and Methods

Seven accessions of mungbeans were planted at Virginia Tech’s Kentland Farm in Blacksburg, Virginia (TABLE 3-1). The preliminary experiment was set up as a randomized split-plot design with four leaf harvest frequencies (LHF) of zero-time leaf harvest (0x, control), one-time leaf harvest (1x), two-time leaf harvest (2x), and three-time leaf harvest (3x). LHF was defined as the whole plot and the seven accessions were defined as the sub-plots with three replications each for a total of 84 plots.

Base fertilizer (P and K) according to soil test results and a pre-emergence herbicide were applied to the field prior to planting. Border plots were planted on the perimeter of the whole field and in between each defoliation treatment. The border was comprised of Berken seed and defoliated in correspondence with adjacent treatment groups in order to maintain consistent ground cover and light penetration. Each plot had two 1.5 m rows with a seeding rate of ~17 seeds per meter and 40 cm interrow spacing. This experiment was repeated for three consecutive years from 2020- 2022 and planted each year between mid-June and early July (**TABLE 3-2**). Approximately two weeks post-planting, the field was weeded manually. If necessary, a post-herbicide was applied.

TABLE 3-1 Mungbean accession descriptors and traits. Adapted from study by Diatta et al. (2018) in Senegal, reviewing overall characteristics of accessions.

Acc.	**Origin Country**	**No. of Leaves**	**TDM**	**Seed Size**	**Seed Yield**	**Plant Habit**	**Seed Color**
			kg ha^{-1}	g 100 seeds^{-1}	kg ha^{-1}		
L1 (8735)	India	34.3	1284	3.24	476	Semi-prostrate	Green
L4 (10693)	India	18.0	853	3.9	1114	Erect	Green
L5 (5)	India	26.9	1143	3.1	1000	Prostrate	Green
L9 (IC 8972-1)	India	17.7	739	3.6	1000	Semi-prostrate	Black
L17 (OLD-TAWING)	Thailand	16.5	617	4.7	707	Semi-prostrate	Yellow
L30 (BK-32, MKT HP 1975)	Thailand	28.4	720	4.4	1763	Prostrate	Green
L35 (Berken) [a]	Australia	--	--	5.1	--	Erect	Green

[a] Berken is the only commercially available cultivar of mungbeans in this trial. No data from Diatta study.

In 2020, only yield components were measured (pods plant^{-1}, seeds plant^{-1}, seeds pod^{-1}, seed size, plant total dry matter (TDM), and plant height). In 2021, all yield components, with the exception of plant height, were measured along with dry leaf yield from all three leaf harvests and grain yield from two grain harvests. In 2022, all yield components, dry leaf yield from three leaf harvests, and grain yield from one grain harvest were measured.

TABLE 3-2 Timetable of planting and harvest for field trials at Kentland Farm, Blacksburg, VA from 2020- 2022.

	LHF	**Weeks post-planting**	**2020**	**2021**	**2022**
Planting Date	0x, 1x, 2x, 3x	Week 0	29 June	6 July	23 June
Leaf Harvest 1	1x, 2x, 3x	Week 7	19 Aug.	2 Sept.	11 Aug.
Leaf Harvest 2	2x, 3x	Week 8	26 Aug.	8 Sept.	18 Aug.
Leaf Harvest 3	3x	Week 9	2 Sept.	15 Sept.	25 Aug.
Grain Harvest 1	0x, 1x, 2x, 3x	Week 13	1 Oct.	6 Oct.	4 Oct.
Grain Harvest 2	0x, 1x, 2x, 3x	Week 14- 15	14 Oct.	14 Oct.	N/A[a]

[a] In 2022, only one grain harvest was collected, and biomass was harvested on the same day as grain harvest 1.

Leaf Harvest Protocol

Each plot was examined around seven weeks post-planting once flowers first began to appear. A selected number of plants were chosen at random and tagged at this time; five plants in 2020 and ten in 2021 and 2022. Only tagged plants underwent different LHFs and were later measured for leaf weight, seed weight, and TDM. The first leaf harvest was completed in week seven for all plots except for the control. The second leaf harvest was completed the following week (week eight post-planting) on only LHF 2x and 3x plots. Finally, the third leaf harvest was completed another week later, week nine post-planting, on only the LHF 3x plots.

The leaves collected during harvest were the fully expanded trifoliate, "young, tender leaves" that were lighter green in color, less rigid, and had softer pubescents than the mature leaves. The young leaves were objectively defined by the leaves which were not directly connected to the main stem by their petioles (FIGURE 3-1). The leaves which grew from branches off the mainstem were harvested. The first leaf on the first branch was also not harvested. The full trifoliate leaf and its petiole were collected. Leaves were collected in paper bags and separated by plot and harvest number.

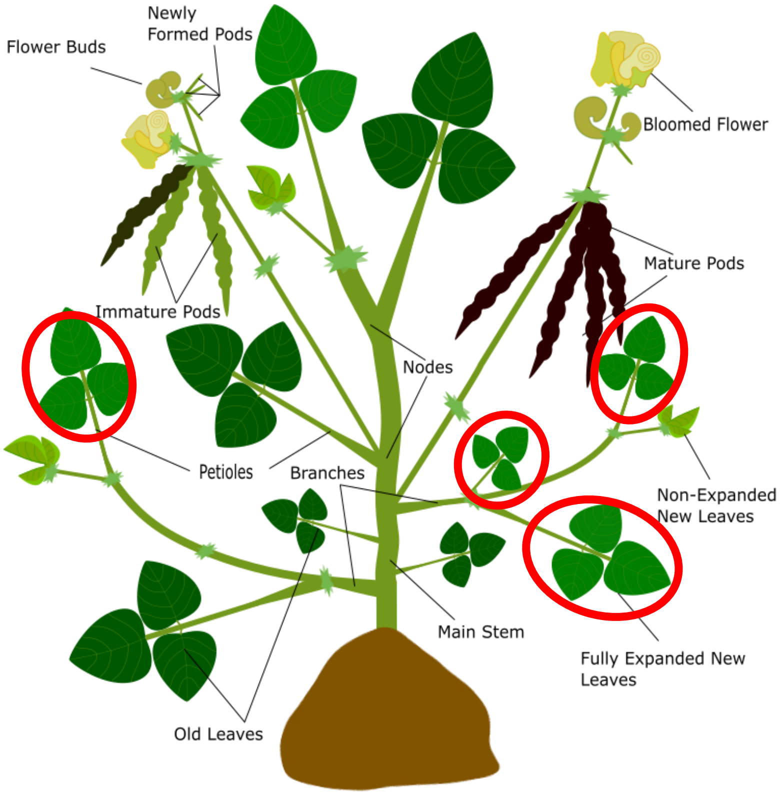

FIGURE 3-1 Leaves removed in leaf harvests. Circled leaves show harvested leaves which are defined by fully expanded trifoliate leaves connected to branches as opposed to the main stem.

Grain Harvest Protocol

The pods of tagged plants were hand harvested when about 75% of the pods were mature and became dark black in color which occurred at thirteen weeks for all three years. Since mungbean has an indeterminant growth habit, a second grain harvest was completed in 2020 and 2021 when nearly all the remaining pods had matured. In 2020, the second grain harvest occurred at week 15 post-planting and in 2021, occurred at week 14 post-planting. In 2022, only one grain harvest was completed due to extremely low yields and weather conditions related to Hurricane Ian. In all years, during the last seed harvest, all of the tagged plants were fully removed from the field and data on plant height (not recorded in 2021) and biomass were collected.

Yield and Yield Component Data Collection

After each leaf and grain harvest, samples were dried in a dryer at 125°F for at least 48 hours or until the leaves and pods were brittle. The dry leaves were weighed for leaf yield per plot (g). Then leaves were ground in a forage grinder and separated into sample cups labeled with plot and leaf harvest number. Leaves from 2021 were sent to University of Missouri labs (https://aescl.missouri.edu) for nutritional analysis. Leaves from the first leaf harvest in 2021 measured crude protein, while the second and third leaf harvest measured crude protein, fat, fiber, and ash. Mature pods were counted for every plot and individually for each grain harvest. The number of seeds was counted per ten representative mature pods to estimate seeds per pod. Seed size was also measured and determined by weighing (g) 100 sample seeds. After all data collection, mature pods were threshed and the seeds were cleaned to measure seed yield per plot.

After the final grain harvest, each plant component: leaves, stems, immature pods, and mature pods was separated from the plant and dried individually. The components were weighed and TDM plant^{-1} was determined by adding the mass (g) of leaves, stems, and immature pods for each plot and dividing by *n* plants. It was estimated that each plot had approximately 17.2 standing and yielding plants by the end of the growing season by randomly selecting ten plots in

2022 in order to calculate TDM, leaf yield and grain yield. The TDM plot^{-1} (g), leaf yield plot^{-1} (g), and grain yield plot^{-1} (g) were converted to kg ha^{-1} by this formula:

$$\frac{\sim 17.2 \text{ plants}}{\text{plot}} \cdot \frac{\text{plot}}{12.5 \text{ ft}^2} \cdot \frac{107639 \text{ ft}^2}{\text{ha}} \cdot \frac{\text{TDM, leaf yield, or grain yield (g)}}{\text{plant}} \cdot \frac{\text{kg}}{1000 \text{ g}}$$

Statistical Analysis

JMP Pro 16 was used to complete the statistical analysis for a split-plot. Year was defined as the block, LHF was the whole plot, and the accession line (acc.) was the sub-plot. A mixed-model ANOVA was run with year*LHF as a random effect, and year, acc., LHF, and acc*LHF as fixed effects. A student t test with a significance value of 0.05 was then used to compare means. For the nutritional analysis, and for grain yield in the second grain harvest, there was only one year's worth of data. This analysis was run with factors, LHF, acc, and LHF*acc as fixed effects and a student t test was performed.

Results

Leaf Yield and Nutrition

In 2021, leaf yields ranged from 0 kg ha^{-1} to 1297 kg ha^{-1} and was highest in the first leaf harvest (659 kg ha^{-1}), second highest in the third leaf harvest (565 kg ha-1), and lowest in the second leaf harvest (220 kg ha^{-1}). Though the range of leaf yield in 2022 (0 kg ha^{-1} to 1281 kg ha^{-1}) was similar to that of 2021, the average leaf yield in the first and second leaf harvests were significantly lower at 79 kg ha^{-1} and 92 kg ha^{-1}, respectively. However, the third leaf harvest (639 kg ha^{-1}) in 2022 yielded over seven-fold that of earlier harvests in the same year (TABLE 3-3). Year was a significant factor in leaf yields for first and second leaf harvests due to the much lower leaf yields in 2022 compared to 2021, but there was no significant difference between leaf yields for 2021 and 2022 in the third leaf harvest. The accession had no significant impact on the overall leaf yields for any of the leaf harvests in either year

TABLE 3-3 Mean, standard deviation, and ANOVA from three leaf harvests in 2021 and 2022.

Effects	**Dry Leaf Yield** **Harvest 1** (LHF 1x, 2x, 3x)	**Harvest 2** (LHF 2x, 3x)	**Harvest 3** (LHF 3x)
		Kg Ha^{-1}	
Year [a]			
2021	659 ± 240 a	220 ± 89 a	565 ± 49 a
2022	79 ± 68 b	92 ± 76 b	639 ± 176 a
Accession Line (Acc)			
L1	333 ± 280 a	151 ± 77 ab	639 ± 97 ab
L4	370 ± 360 a	136 ± 107 b	582 ± 112 ab
L5	389 ± 340 a	208 ± 100 a	552 ± 48 b
L9	378 ± 388 a	149 ± 102 ab	581 ± 79 ab
L17	334 ± 331 a	160 ± 127 ab	583 ± 69 ab
L30	424 ± 410 a	127 ± 113 b	565 ± 102 b
L35	354 ± 303 a	159 ± 105 ab	722 ± 281 a
Mean ± SD	369 ± 340	156 ± 104	603 ± 135
Significance		*P > f*	
Year	<.0001**	<.0001**	ns
Acc	ns	ns	ns
Year*Acc	ns	ns	ns

Note. Within columns and effect group, means followed by the same letter are not significantly different according to student t test (.05).

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] No leaf yield data was measured in 2020.

Nutritional composition of harvested leaves was analyzed for 2021 samples, and year was not an effect. Crude protein ranged from 14.11% in LHF 3x leaves to 26.71% in LHF 1x leaves and had an overall average of 22.0% protein. LHF had a significant effect on leaf crude protein with LHF 3x being significantly lower than LHF 1x and 2x. Accession also affected crude protein with L9 (23.8%) being significantly higher than L1 (21.1%) and L17 (20.7%) (TABLE 3-4).

TABLE 3-4 Mean, standard deviation, and ANOVA for leaf nutrition in 2021 samples.

	Leaf Nutrition [a]				
Effects	**Crude Protein**	**Moisture**	**Crude Fat**	**Crude Fiber**	**Ash** [c]
			W/W%		
Leaf Harvest Frequency (LHF)					
1x [b]	22.2 ± 2.1 ab	-	-	-	-
2x	22.6 ± 2.2 a	9.4 ± 0.4 a	1.9 ± 0.4 a	12.4 ± 1.4 a	8.9 1.3
3x	20.3 ± 3.0 b	8.7 ± 0.7 b	1.8 ± 0.4 a	12.0 ± 3.2 a	7.1 0.8
Accession Line					
L1	21.1 ± 2.7 c	9.3 ± 0.6 ab	1.9 ± 0.5 ab	13.1 ± 3.2 ab	7.3 1.2
L4	22.8 ± 2.1 ab	9.3 ± 0.5 ab	1.9 ± 0.4 ab	12.2 ± 0.7 ab	9.2 1.6
L5	21.2 ± 2.4 bc	9.3 ± 0.6 a	1.7 ± 0.4 ab	12.9 ± 1.1 ab	8.4 0.6
L9	23.8 ± 2.4 a	9.1 ± 0.6 ab	2.0 ± 0.5 a	11.7 ± 1.6 a	8.2 0.9
L17	20.7 ± 1.9 c	9.0 ± 0.6 ab	1.6 ± 0.3 b	10.8 ± 2.2 b	8.2 0.8
L30	23.4 ± 1.9 ab	9.3 ± 0.8 b	1.8 ± 0.4 ab	12.4 ± 1.2 ab	8.9 0.6
L35	20.9 ± 2.7 abc	9.3 ± 0.6 ab	2.0 ± 0.2 ab	12.6 ± 0.9 ab	10.5 1.9
Mean ± SD	22.0 ± 2.5	9.2 ± 0.6	1.8 ± 0.4	12.3 ± 1.9	8.5 1.4
Significance			*P > f*		
LHF	0.0291*	<.0001**	ns	ns	n/a
Line	0.0349*	ns	ns	ns	n/a
Line*LHF	ns	ns	ns	ns	n/a

Note. Within columns and effect group, means followed by the same letter are not significantly different according to student t test (.05).

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] Leaf nutrition only reported in 2021.

[b] LHF 1x only measure crude protein in leaf nutrition.

[c] Insufficient amount of leaf sample in leaf harvest 3, limited ash analysis and loss DFs, making LHF 2x and LHF 3x incomparable.

Moisture, crude fat, crude fiber, and ash were only measured for LHF 2x and 3x in 2021 leaf samples. Moisture ranged from 7.58% in LHF 3x to 10.10% in LHF 2x and had an average of 9.2%. LHF 2x was significantly higher than LHF 3x, but there was no significant difference between cultivars. Crude fat had a minimum content of 0.93% and a maximum content of 2.73% with an average of 1.83%. Crude fiber ranged from 8.69% in LHF 3x to 14.95% in LHF 2x with an outlier of 20.74% in LHF 3x and an average of 12.4%. Neither crude fat nor crude fiber had any significant effects. Ash was analyzed but no significance test was performed due to insufficient sample and lost degrees of freedom. However, it was found that ash content ranged from 5.3% to 12.99% and had an average of 8.5%. The accession with the lowest ash content was L1, while Berken had the highest. LHF 3x had a higher average ash (7.1%) than LHF 2x (8.9%) though it was not statistically analyzed.

Grain Yield

Grain yield was measured for the first grain harvest in 2021 and 2022. In 2021, grain yield ranged from 0 kg ha^{-1} to 1629 kg ha^{-1} and had an average of 545 kg ha^{-1}. While in 2022 for the first grain harvest, yield had a minimum of 0 kg ha^{-1}, a maximum yield of 594 kg ha^{-1} and an average yield of only 101 kg ha^{-1}. Because of this, year was a significant effect on grain yield for the first grain harvests. Accession also had a significant effect on grain yield with L35 producing the highest yield (39 to 1629 Kg ha^{-1}) and L1 producing the lowest yield (0 to 490 Kg ha^{-1}) (TABLE 3-5). Though there was no significant difference between LHFs, both LHF 1x and 2x yields were higher than the control treatment, LHF 0x.

TABLE 3-5 Mean, standard deviation, and ANOVA of grain yield and yield components for the first seed harvest in 2020- 2022 trials.

	First Grain Harvest			
Effects	**Pods 10 Plants^{-1}**	**Seeds Pod^{-1}**	**Seed Size**	**Grain Yield [a]**
			g 100 seeds^{-1}	Kg Ha^{-1}
<u>Year</u>				
2020	93.9 ± 143.9 a	11.3 ± 1.6 a	5.1 ± 1.1 a	-
2021	73.7 ± 45.7 b	11.6 ± 1.2 a	4.6 ± 1.1 b	545 ± 362 a
2022	18.3 ± 23.3 c	11.6 ± 1.4 a	3.7 ± 0.8 c	101 ± 129 b
<u>Leaf Harvest Frequency (LHF)</u>				
0x	52 ± 70.7 b	11.5 ± 1.3 a	4.5 ± 1.2 a	281 ± 309 a
1x	69.5 ± 99.5 a	11.4 ± 1.5 a	4.4 ± 1.1 a	375 ± 340 a
2x	75.3 ± 114.9 a	11.7 ± 1.4 a	4.5 ± 1.3 a	385 ± 428 a
3x	50.9 ± 82.9 b	11.5 ± 1.4 a	4.4 ± 1.1 a	250 ± 305 a
<u>Accession Line (Acc)</u>				
L1	25.2 ± 42.3 c	10.8 ± 1.9 c	3.2 ± 0.7 d	96 ± 133 d
L4	54.4 ± 62.6 b	11.0 ± 1.3 bc	3.8 ± 0.6 c	373 ± 379 b
L5	59.2 ± 66.0 bc	11.7 ± 0.9 b	4.0 ± 0.6 c	297 ± 142 bc
L9	22.7 ± 30.0 c	11.8 ± 1.0 b	4.1 ± 1.1 c	183 ± 213 cd
L17	94.7 ± 110.7 b	11.3 ± 1.0 bc	5.5 ± 0.7 a	411 ± 321 b
L30	29.3 ± 53.2 c	11.1 ± 1.6 bc	5.0 ± 0.92 b	225 ± 343 c
L35	145.6 ± 154.7 a	12.5 ± 0.8 a	5.3 ± 1.2 a	659 ± 479 a
Mean ± SD	62.0 ± 93.5	11.5 ± 1.4	4.4 ± 1.2	323 ± 351
Significance			*P > f*	
Year	<.0001**	ns	<.0001**	<.0012**
LHF	0.0067**	ns	ns	ns
Acc	<.0001*	<.0001**	<.0001**	<.0001**
Acc*LHF	ns	ns	ns	ns
Year*LHF	ns	ns	ns	ns

Note. Within columns and effect group, means followed by the same letter are not significantly different according to student t test (.05).

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] Grain yield was not reported for 2020.

Pods per Plant

In the first grain harvest, it was found that pods per plant were significantly higher in 2020 (0 to 322 pods 10 plants^{-1}), second highest in 2021 (0 to 213 pods 10 plants^{-1}), and lowest in 2022 (0 to 113 pods 10 plants^{-1}) (TABLE 3-5). Meanwhile, pods per plant for the second grain harvest was not affected by year and ranged from 0 to 288 pods 10 plants^{-1} in 2020 and from 10 to 177 pods 10 plants^{-1} in 2021. LHF also did not affect pods per plant in second grain harvest, but LHF was effective in the first grain harvest. First grain harvest found that LHF 1x and 2x increased pods per plant over the control by 17.5 and 23.3 pods 10 plants^{-1}, respectively. Accession had a significant effect on pods per plant in both the first and second grain harvests, but in the second

TABLE 3-6 Mean, standard deviation, and ANOVA of grain yield and yield components for the second grain harvest in 2020 and 2021 trials.

	Second Grain Harvest			
Effects	**Pods 10 Plants^{-1}**	**Seeds Pod^{-1}**	**Seed Size**	**Grain Yield [a]**
			g 100 seeds^{-1}	Kg Ha^{-1}
Year [b]				
2020	86.3 ± 65.5 a	11.6 ± 1.7 a	4.52 ± 1.1 a	-
2021	73.7 ± 43.1 a	11.4 ± 1.2 a	4.0 ± 0.9 b	402 ± 232
Leaf Harvest Frequency (LHF)				
0x	78.7 ± 57.1 a	11.3 ± 1.5 a	4.5 ± 1.1 a	485 ± 310 a
1x	80.6 ± 57.4 a	11.7 ± 1.8 a	4.13 ± 1.0 bc	367 ± 196 ab
2x	73.0 ±48.2 a	11.6 ± 1.3 a	4.4 ± 1.0 ab	435 ± 209 a
3x	86.6 ± 59.1 a	11.5 ± 1.1 a	4.0 ± 1.0 c	320 ± 170 b
Accession Line (Acc)				
L1	69.7 ± 40.4 bcd	12.2 ± 1.3 a	3.4 ± 0.9 c	380 ± 190 ab
L4	107 ± 42.3 a	11.2 ± 0.9 bc	3.7 ± 0.6 c	482 ± 193 a
L5	87.6 ± 45.6 abc	11.6 ± 0.9 ab	3.6 ± 0.8 c	437 ± 125 a
L9	64.1 ± 48.7 cd	11.2 ± 1.3 bc	3.6 ± 0.3 c	512 ± 194 a
L17	50.3 ± 40.7 d	10.7 ± 1.7 c	5.3 ± 0.5 a	244 ± 136 b
L30	99.9 ± 78.0 ab	11.8 ± 1.8 ab	4.8 ± 1.0 b	539 ± 403 a
L35	77.2 ± 65.6 bc	12 ± 1.5 a	5.1 ± 0.8 ab	245 ± 121 b
Mean ± SD	79.9 ± 55.3	11.5 ± 1.4	4.2 ± 1.0	402 ± 232
Significance			*P > f*	
Year	ns	ns	0.0039**	-
LHF	ns	ns	ns	0.0220*
Acc	0.0033*	0.0036*	0.0001**	0.0001**
Acc*LHF	ns	ns	ns	0.0027**
Year*LHF	0.0135*	ns	ns	-

Note. Within columns and effect group, means followed by the same letter are not significantly different according to LSM (05).

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] grain yield for second grain harvest was not reported for 2020.

[b] Only years 2020 and 2021 had second grain harvests, 2021 had one grain harvest.

harvest, the interaction between accession and LHF was also significant. This means that the accessions with the highest and lowest number of pods per plant differed between the first and second. In the first seed harvest, L35 had significantly higher pods per plant ranging from 0 to 322 pods 10 plants^{-1} than all other accessions, but in the second harvest, L35 was fourth highest ranging from 18 to 232 pods 10 plants^{-1}. Similarly, L17 (94.7 pods 10 plants^{-1}) was statistically higher in pods per plant for the first grain harvest than four other accessions but was statistically lowest in the second grain harvest (50.3 pods 10 plants^{-1}). In general, the second grain harvest had more pods per plant with an average of 79.9 pods 10 plants^{-1} compared to the first harvest with 62.0 pods 10 plants^{-1}.

Seeds per Pod

The number of seeds per pod was consistent in both grain harvests as well as across all years with both harvests having an average of 11.5 seeds pod^{-1} with standard deviations of 1.4. Therefore, the year and LHF did not have a significant effect on the number of seeds per pod, but there was significant variation across accessions. In the first grain harvest, L35 had the highest number of seeds per pod ranging from 9.5 to 13.9 seeds pod^{-1} while L1 had the lowest number of seeds per pod ranging from 6.1 to 13.4 seeds pod^{-1} (TABLE 3-5). In the second harvest, L35 (9.8 to 16.8 seeds pod^{-1}) had significantly the highest seeds per pod, but surprisingly, L1 (8.5 to 14.3 seeds pod^{-1}) was also significantly highest (TABLE 3-6).

Seed Size

Seed size when averaged over all accessions for the first grain harvest was significantly largest in 2020 (5.1 g 100 seed^{-1}), followed by 2021 (4.6 g 100 seed^{-1}) and then 2022 (3.7 g 100 seed^{-1}). Second grain harvest was also significantly impacted by year and followed a similar trend with 2020 (4.5 g 100 seed^{-1}) having the highest seed size followed by 2021 (4.0 g 100 seed^{-1}).

Accession had a significant effect on seed size and was similar in both first and second grain harvests. L35 (5.2 g 100 seed^{-1}) and L17 (5.4 g 100 seed^{-1}) had the largest seed sizes in both harvests while L30 (4.9 g 100 seed^{-1}) was the second largest followed by the remaining accessions (3.8 g 100 seed^{-1}).

Plant Height & TDM

Plant height ranged from 73.2 cm to 197.1 cm in 2020 and 2021 with an average height of 121.0 cm. LHF and year had no significant effect on plant height. The accession, however, did have a significant effect. L9 was significantly the tallest accession ranging from 135.5 cm to 197.1 cm and L35 was the shortest accession at 73.2 cm to 118 cm (TABLE 3-7). TDM was significantly lowest in 2020 at 2388 kg ha^{-1}, but similar in 2022 at 3264 kg ha^{-1} and 2021 at 3045 kg ha^{-1}. LHF had no effect on TDM, but accession did make a significant impact. Plant height had little correlation to TDM since the accessions with the highest TDM were L4 ranging from 1113 kg ha^{-1} to 4765 kg ha^{-1} and L1 ranging from 1318 kg ha^{-1} to 5043 kg ha^{-1}. The accession with the smallest TDM was L17 ranging from 908 kg ha^{-1} to 3352 kg ha^{-1}.

TABLE 3-7 Mean, standard deviation, and ANOVA of plant height and TDM for the final plant harvest in 2020 -2022 trials.

	Final Plant Harvest [a]	
Effects	**Plant Height** [b]	**Total Dry Matter Yield**
	cm	Kg Ha^{-1}
Year		
2020	120.7 ± 27.6 a	2388 ± 1090 b
2021	-	3264 ± 581 a
2022	121.3 ± 22.4 a	3045 ± 978 a
Leaf Harvest Frequency (LHF)		
0x	124.5 ± 20.9 a	2698 ± 775 a
1x	124.5 ± 28.8 a	2977 ± 885 a
2x	117.1 ± 26.9 a	3089 ± 1203 a
3x	117.6 ± 22.6 a	2825 ±986 a
Accession Line (Acc)		
L1	125.6 ± 13.5 b	3146 ± 893 a
L4	125.6 ± 14.4 b	3044 ± 883 ab
L5	125.2 ± 15.6 b	3152 ± 929 a
L9	161.8 ± 16.5 a	2978 ± 766 ab
L17	106.5 ± 9.4 c	2289 ± 913 c
L30	105.8 ± 24.0 c	2972 ± 1006 ab
L35	96.5 ± 9.7 d	2713 ± 1203 b
Mean ± SD	121.0 ± 25.0	2897.6 ± 980.6
Significance	*P > f*	
Year	ns	0.0192*
LHF	ns	ns
Acc	<.0001**	<.0001**
Acc*LHF	ns	ns
Year*LHF	ns	ns

Note. Within columns and effect group, means followed by the same letter are not significantly different according to LSM (05).

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] Final plant harvest was completed on the same day as last bean harvest. In 2020 and 2021, that was in week 14 and in 2022 was in week 13.

[b] Plant height not reported for 2021.

Discussion

In Blacksburg, Virginia, the climate was consistent during the growing season from 2020 to 2022. The cumulative precipitation was 399 mm, 413 mm, and 406 mm in 2020, 2021, and 2022, respectively. While the average low temperature was 15.7˚C, 15.8˚C, and 16.2˚C and the average high temperature was 25.9˚C, 27.0˚C, 26.3˚C in 2020, 2021, and 2022, respectively (FIGURE

3-2). Though the climate in Blacksburg meets optimal water conditions for mungbean between 350 to 550 mm, the temperature often dropped below the optimal 25˚C (AVRDC The World Vegetable Center, 2016).

In Iowa, mungbean height typically ranges from 60- 76 cm (Myers, 2003) but in our study, plant height ranged from 73 cm to 197 cm with an average height of 122 cm. The growth type was vine-like and all of the mungbeans lodged. Overall plant biomass was also relatively high ranging from 608 kg ha^{-1} to 6781 kg ha^{-1} with an average of 2909 kg ha^{-1}. Meanwhile, seed yields had a low average of 362 kg ha^{-1} compared to global production yields which usually ranges from 500 to 1500 kg ha^{-1} (Nair & Schreinemachers, 2020). The high vegetative growth and low bean yields could be related to lower than optimal temperatures and near maximum water levels, resulting in high soil moisture driving the plant to allocate more of the plant's assimilates to vegetation production.

Though climate was similar across all years, there were drastic differences between 2021 and 2022 in yields from leaf harvests one and two. Seed size and yield in both seed harvests and pods per plant in the first seed harvest were also significantly affected by year. In these cases, the yield or yield components decreased with each consecutive year. Since environmental conditions and management practices were relatively constant with little sign of yield-reducing plant diseases, we hypothesize that the seed quality declined with each passing year. Germination tests were run before each planting year with a percent germination 95% or higher and there was no obvious germination problems in the field in any given year. Seed size, though affected by genetics, can also be a good indicator of seed quality (Lehtilä & Ehrlén, 2005). When averaging the seed size of all seven genotypes to account for genetic variation, seed size significantly decreased from 5.1 g 100 seeds^{-1} to 4.6 g 100 seeds^{-1} in 2021 to 3.7 g 100 seeds^{-1} in 2022. In

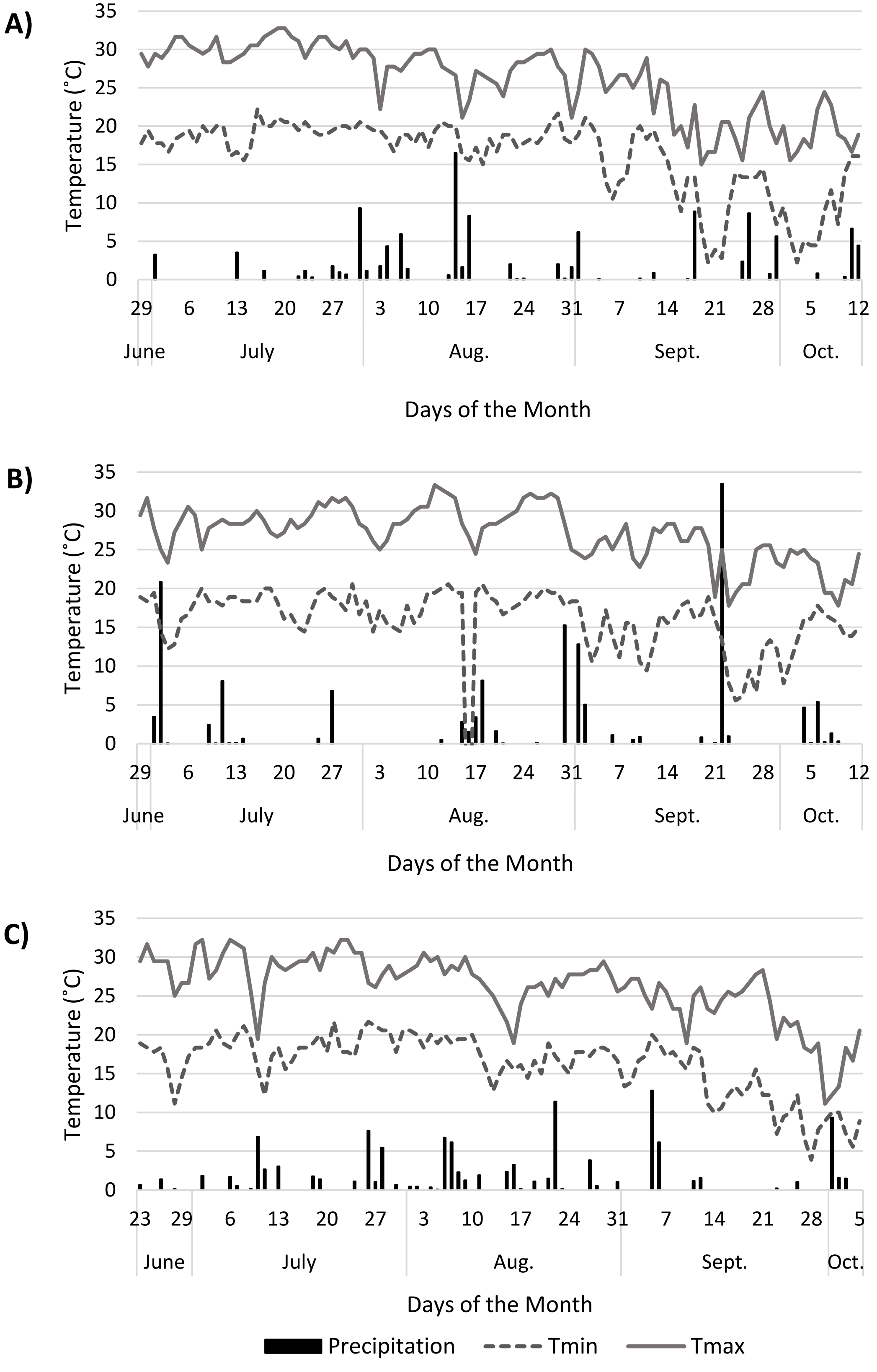

FIGURE 3-2 Blacksburg Climatographs during growing seasons in 2020 **(A)**, 2021 **(B),** and 2022 **(C)**.

2020, seed was used from the USDA GRIN germplasm bank whereas in following years, seed was used from the previous year's harvest. Though seed was cleaned before planting, due to less-than-ideal growing conditions, seed quality likely declined with each passing year.

Across all leaf harvests in 2021, leaf yield added to be an average of 1,444 kg ha^{-1}. There was also no difference in leaf yield between accessions in any year. Plant growth appeared to be more stunted in earlier vegetative stages in 2022. This may be in response to poorer seed quality in 2022. The stunted growth resulted in fewer branches on plants in 2022 during the vegetative growth stages of the plants. Because the harvested leaves were defined as "fully-expanded trifoliate leaves connected to the branches and not the main stem," the lack of branches during the first and second harvest resulted in many plots having little to no leaves harvested. The average leaf harvest for the first and second harvests was only a combined yield of 171 kg ha^{-1} compared to the combined yield in 2021 of 879 kg ha^{-1}. By week nine of 2022, the time of the third leaf harvest, the leaf yield was seven times higher than earlier leaf yields for an average of 639 kg ha^{-1}. This shows that the plants initially took longer to develop and produce branches in 2022 compared to in 2021, but that by week nine, the plants were still able to produce a high leaf yield. A study in 2011 showed that removing the top 25- 75% of mungbean leaves can increase overall number of branches (Mondal et al., 2011). The removal of some leaves in the first and second harvests may have driven the plant to produce more branches, but more investigation would have to be done to conclude.

The overall nutrition of mungbean leaves was measured after each LHF treatment. Crude protein was significantly higher after LHF 1x (22.2%) and 2x (22.6%) then 3x (20.3%). It is likely that the protein content of the leaf reduced after LHF 3x because it was harvested later in the growing season and the plant is allocating more energy into filling pods and less into the leaves. Crude fat, crude fiber, and ash were unaffected by LHF and had 1.8%, 12.3%, and 8.5%, respectively. A study in eastern Nigeria measured the nutrient content of cowpea leaves when the tender leaves are harvested at 8 weeks for human consumption and found that at a moisture of 10.88%, cowpea has a protein content of 34.91%, fiber content of 19.46%, fat content of 5.42%, and ash content of 11.15% (Enyiukwu et al., 2018). In another study replicated in Ghana and South Africa, 32 genotypes of cowpea were analyzed for leaf protein and found that leaf protein ranged from 23% to 40% based on genotype (Dakora & Belane, 2019). Though the average protein and nutrient content in mungbean is slightly lower in mungbean than reported in cowpea, mungbean leaves still have a comparable amount of protein, fiber and ash under all LHFs.

LHF had no significant effect in years 2020 to 2022 on grain yield during the first harvest. This means that plants can undergo leaf harvest at least two times and have no negative impact on overall grain yield. When grain was harvested a second time in 2020 and 2021, LHF did have a significant effect and yield was significantly lowered when leaves were harvested three times, but not when harvested one and two times in comparison to the control. Ultimately, harvesting up to two times for leaves, did not reduce yield for the first or second grain harvest. Yield components were also not significantly affected by any level of leaf harvest except for pod per plant in the first seed harvest. In this case, LHF 1x and 2x actually improved the number of pods per plant over the control.

This study was replicated at the Universite Assaine Seck in Ziguinchor, Senegal in 2020 (Awa, 2021). The results of that study validate the results of this study. It was found that grain yield (1982 to 2409 kg ha-1), pods per plant (16.5 to 22.1), and seed size (10.2 to 11.2 g 100 seeds-1) were not affected by LHF. Also, leaf yield averaged to be 1715 kg ha-1 at the end of the third leaf harvest.

Conclusion

This study found that mungbean leaves can be harvested up to two times without significantly decreasing overall grain yield and allows for multiple grain harvests in one season. The leaves are highly nutritious with an average crude protein content of 22.0% (in dry weight), 1.8% crude fat, 12.3% crude fiber, and 8.5% ash. Given that mungbeans fully mature in ten to thirteen weeks, harvesting for leafy greens starting at week seven could allow the first crop three to five weeks prior to grain harvest. In Senegal, mungbean has the potential to be used as a dual-purpose crop, help improve dietary diversity, and provide vital minerals and nutrients to help combat the endemic of anemia.

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Chapter 4. Introducing Mungbean as an alternative crop to Tobacco in Virginia

Introducing Mungbean as an Alternative Crop to Tobacco in Virginia

Jessica Wilbur

ABSTRACT

Mungbean [*Vigna radiata* (L.) R. Wilczek] is an important grain legume crop in Asia, particularly in India. Mungbean is consumed in the United States by a niche market in Asian cuisines, mostly as bean sprout, but is on the rise for use as an alternative plant protein due to its high protein, micronutrients such as zinc, iron, and folic acid, and low fat. Currently, more than two-thirds of mungbeans consumed in the United States are imported and nearly all domestically farmed mungbeans are produced in Oklahoma. This project explores the viability of mungbean to be produced by Virginia tobacco farmers as an alternative specialty crop since the demand for tobacco is on the decline. Two cultivars commercially available in the United States, Berken and OK 2000, were planted in split-plot design in three locations each year in 2021 and 2022 in southern piedmont Virginia. The seeds were sown with early and late planting dates in June and July in farmers' fields and Virginia Tech's Southern Piedmont Agricultural Research and Extension Center. Results were analyzed using a one-way ANOVA in SAS for individual locations and years. Due to highly variable rain patterns in 2021 and 2022, as well as differing management practices, there were no consistent effects of genotype or planting date on yield, plant height, pods per plant, seeds per pod, or seed size. Yield ranged from 0.19 MT ha^{-1} to 1.18 MT ha^{-1} with an average yield of 0.84 MT ha^{-1} in 2021 and 0.38 MT ha^{-1} in 2022. The highest yield is above the global average and the average yield between both years is higher than the average yield of India, the largest mungbean producer in the world. There are still many challenges to production in Virginia such as developing a proper production guide, breeding for determinant and non-lodging varieties adapted to Mid-Atlantic region, and the need for a supply

chain. Our results suggest that there is great potential for mungbean in Virginia, but investments must be made into the production and research of this crop.

Introduction

Mungbean [*Vigna radiata* R. Wilczek] is a warm-season, pulse crop in the Fabaceae family native to India and consumed across Asia. Mungbean's most notable production characteristics are its short growth cycle which matures in 60-90 days, depending on the variety, and its drought tolerance. Compared to corn, mungbean requires 2-2.5 times less water to produce the same amount of acreage (Amarasingha et al., 2017). Mungbean is best produced in silty loam soil which results in the highest soil nitrogen uptake and plant dry matter (Diatta et al., 2020b) but can suffer under high-salinity soils (Ahmad et al., 2013; Barkodia et al., 2017) and waterlogged soils (Kumar et al., 2013). Similarly, to other legumes, mungbean is high in isoflavonoids, antioxidants, amino acids, minerals, protein, and vitamins which are associated with health benefits such as combatting hyperglycemia, hyperlipemia, and hypertension, and has cancer prevention properties (Akpapunam, 1996; Bessada et al., 2019; Hou et al., 2019).

In the United States, there is a market in Asian cuisine for mungbean as a specialty crop (Fery, 2002). In Chinese and Korean dishes, mungbean is one of the most important beans used for bean sprouts. Mungbean dahl which is the split bean boiled down into a stew-like meal is very important to South Asian cuisine (Lambrides & Godwin, 2007). Mungbean is a common pulse sold at Asian markets in the United States because of this niche market in Asian cuisine, but nearly all mungbeans that are sold are imported from India, China, and Thailand (USDA, 2022). Imported mungbeans increased in value from 2.84 million USD in the 2018/19 market year to 6.00 million USD in 2020/21 market year for a 111% increase. In 2020/21, 36,000 MT of mungbeans were imported while only 2,600 MT were exported (USDA, 2022).

Although there is a US market for mungbean in traditional Asian dishes, the recent increase in demand for mungbean in the United States is mostly due to its use in plant-based meat and egg alternative products. Products such as Beyond Burger and JUST Egg use mungbean as the main protein isolate and can be found in major grocery stores (Beyond Beef; JUST Egg). Demand for plant alternative proteins is expected to continue to increase in the United States as consumers are making a push for more sustainable food options (Havlík et al., 2014). The alternative plant protein industry is projected to reach 21.23 billion USD globally by 2025 (de Boer et al., 2014). Despite large domestic and international market opportunities, mungbean production is limited in the US and is mainly grown in Oklahoma with some grown in California, Texas, Tennessee, and Kentucky. Research to expand mungbean and breeding efforts for Iowa are underway (Arti Singh et al., 2021).

A study by Bhardwaj and Hamama in 2015 found that mungbean could be a successful crop in Virginia with yields ranging from 1.15-1.47 MT ha^{-1}. Mungbean yields on average are 1.13 MT ha^{-1} but can reach as high as 1.90 MT ha^{-1} (Chauhan & Williams, 2018; Thomas et al., 2004). Mungbean also has the potential to be double-cropped with winter wheat instead of soybean because of its short growth cycle. Double-cropped soybean typically loses about half a bushel per acre in Virginia due to its late planting date and the plant's inability to fully develop its leaf canopy for light absorbance (Holshouser, 2014). On the contrary, mungbean would have time for full development after the harvest of winter wheat in late June or early July and before the first frost.

Since 1965, tobacco usage has declined drastically in the United States from 43% of the population smoking cigarettes to 18% in 2012 (Warren et al., 2014). When including cigarettes and alternative tobacco products such as e-cigarettes, cigars, and smokeless tobacco, 19% of adults in the United States reported using at least one tobacco product in 2020 (Cornelius et al., 2022). This decline in tobacco demand has resulted in a decrease of grown tobacco from 931,655 acres in 1982 to 331,552 acres in 2017 in the United States (USDA, 2017). In Virginia, specifically, tobacco has decreased from 53,770 acres in 1997 to 23,039 acres in 2017 (USDA, 2017). Since the decline of tobacco, many Virginia farmers have opted to grow more agronomic crops such as corn, soybean, and wheat as well as specialty crops such as hemp (McDonough, 2022). This study seeks to find the potential of mungbean as an alternative crop to tobacco growers and its ability to fit into current crop rotations in Virginia. The objectives of this study are to determine the effect of cultivars, planting date, and production management on mungbean yields and yield components in order to validate mungbean as an alternative crop in Virginia.

Materials and Methods

Cultivars

Berken and OK 2000, two commercially available cultivars of mungbean were used at several locations in Southside Virginia. The mungbean varieties, Berken and OK 2000 are medium-large with bright green seed coats. Seeds were purchased from the Oklahoma Seed Foundation and inoculated with cowpea *bradyrhizobia* purchased from Amazon.

Locations

Field tests were conducted in farmers' fields (Brick Goldman's farm in Cullen in 2021 and Red Handle Farm in Amelia Courthouse in 2022) in southern Virginia and at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center (SPAREC) each year. Both farms are smaller-scale vegetable producers. SPAREC is a research station primarily used for tobacco and field crop production.

Experimental Design

Field tests were conducted as a strip plot with the planting date as the vertical strip and cultivars as the horizontal strip. There were two planting dates, early and late (TABLE 4-1) and two cultivars. Each treatment group was replicated three or four times, depending on the size of the field available. Plots were planted in four rows, each 7.62 m long and with 38.1 mm or 76.2 mm interrow spacing at a 2-3 cm planting depth (TABLE 4-1). The seeding rate for the 76.2 mm rows was 95,680 seeds ha^{-1} while for the 38.1 mm rows, the seeding rate was 191,360 seeds ha^{-1}. The selected seeding rate resulted in every row having 56 seeds planted, regardless of row spacing. Red Handle Farm only had three replications and 38.1 mm interrow spacing due to the smaller field.

TABLE 4-1 Planting Dates (PD) and Harvest Dates (HD) at SPAREC, Goldman Farm, and Red Handle Farm in 2021 and 2022.

	Farm Name	**No. of Reps**	**Row Space**	**Early PD**	**Late PD**	**Early HD**	**Late HD**
2021	SPAREC	4	76.2 mm	Jun. 2	Jun. 21	Sept. 27	Oct. 4
	Goldman Farm	4	76.2 mm	Jun. 16	Jun. 28	Oct. 4	Oct. 4
2022	SPAREC	4	76.2 mm	Jun. 17	Jul. 1	Sept. 28	Sept. 28
	Red Handle Farm	3	38.1 mm	Jun. 17	Jul. 1	Sept. 14	Sept. 22

Field Cultivation and Management

Management practices varied from field to field since the upkeep of the mungbean trials were primarily the farmer’s responsibility.

The fields at SPAREC in 2020 and 2021 were disk tilled and 0-6-18 fertilizer was applied at 50 kg ha^{-1} to prepare the soil. Mungbeans were planted using a four-row soybean planter and an electric fence surrounded the field to prevent deer damage. In 2022, plots were irrigated with one inch of water immediately after planting, but not in 2021. In both years, the herbicide Poast was sprayed in late July to control weeds. In 2022, Poast was applied a second time with Sharpen two weeks prior to harvest. Sharpen is a food-safe dry bean desiccant that was sprayed with the intention of being able to mechanically harvest. There were too many green leaves on the plants after spraying with Sharpen to combine and plots were hand harvested in both years.

In 2021, the Goldman Farm was prepared by applying 6.2 L ac^{-1} of Prowl for weed prevention, disking and applying 19-19-19 fertilizer. The mungbeans were planted using a hand planter and rain-fed. Following seedling emergence of both planting dates, 6.2 L ac^{-1} of Poast was sprayed to help manage weed pressure. In 2022, Red Handle Farm tilled the planting area and applied fertilizer based on soil test recommendations for soybean and the mungbeans were planted by hand. After plant establishment, the field was hand weeded periodically until about halfway throughout the growing season due to higher demands from other crops. The second planting date, therefore, was not hand weeded as frequently and therefore suffered from high weed pressure. No herbicides or irrigation was applied throughout the growing season. At both Red Handle Farm and the Goldman Farm, the plots were hand harvested.

Harvest and Data Collection

Harvest time was determined when 75% of pods were mature, or when the green pod turned completely dark black. Five representative plants were selected from two center rows for estimation of yield components: plant height, pods per plant, seeds per pod, and seed size. Plant height (cm), measured in the field, was taken from the base of the plant to the highest terminal bud. After the five plants were selected, the mature pods were harvested by hand from the remaining plants in the two center rows.

Pods per plant were determined by counting the total number of mature pods on all five plants and dividing by five. Seeds per pod were determined by selecting a total of ten representative pods from any of the five plants, counting the number of seeds and dividing by ten. Seed size (g 100 seeds^{-1}) was measured by weighing 100 seeds from the selected plants. The weight of seeds from the five plants was added to the harvested plot for yield calculation.

Statistical Analysis

Statistical analysis was conducted using SAS version 9.4 using PROC ANOVA. Each location was analyzed independently due to variations in field management, planting dates, and harvest dates. Fisher's Least Significant Difference (LSD) at a significance level of $P<0.05$ was used to determine treatment differences.

Results and Discussion

Yield

The yield ranged from 0.35 (OK 2000 in late planting) to 1.04 MT ha^{-1} (Berken in late planting) with an average yield of 0.80 MT ha^{-1} (FIGURE 4-1) at SPAREC in 2021. There were no significant differences between planting dates or cultivars for yield. The only significant interaction between planting date and cultivar was for yield (TABLE 4-2). Late-planted Berken was higher yielding than early-planted Berken whereas early-planted OK 2000 was higher yielding than late-planted OK 2000. The interaction of planting date and cultivar was significant because for Berken, late planting was more advantageous, while for OK 2000, early planting was optimal (FIGURE 4-1).

Yield ranged from 421 (OK 2000 in late planting) to 1.13 MT ha^{-1} (OK 2000 in early planting) with an average yield of 0.88 MT ha^{-1} at the Goldman Farm in 2021 (FIGURE 4-1). There were no significant differences between planting dates and between cultivars for yield (TABLE 4-2). OK 2000 had a large variation for both planting dates with a standard deviation of 0.15 in early planting and 0.14 MT ha^{-1} in late planting. Berken in early planting also had a wide range of yields with a standard deviation of 0.16 MT ha^{-1} (FIGURE 4-1). Though early-planted OK 2000 had the highest yield average, followed by early-planted Berken, the variation in replications resulted in a lower certainty of the significance of planting date.

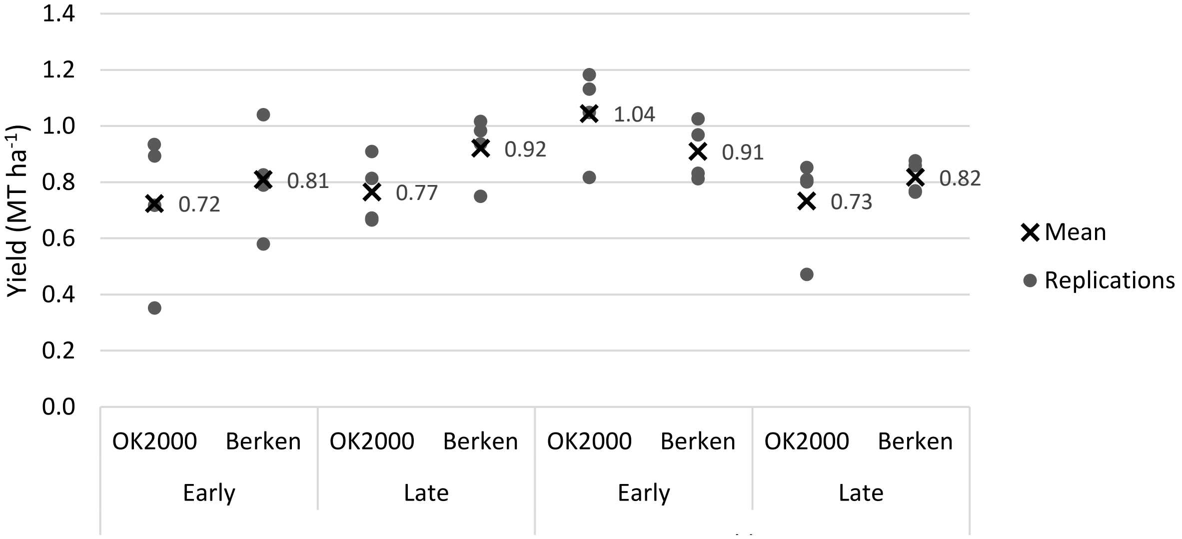

FIGURE 4-1 Spread and means of yield data from all replications of OK 2000 and Berken in late and early plantings at both locations in 2021.

The average yield across planting dates and cultivars in 2021 for SPAREC and the Goldman Farm were similar at 0.80 and 0.88 MT ha^{-1}, respectively. Though mungbean is a drought-tolerant crop, water after the initial planting is important for germination and stand establishment. Mungbean is also most productive on silty loam soil, then sandy loam, and least productive on clayey soil (Diatta et al., 2020a) which has a higher water holding capacity and is more prone to waterlogging. In 2021, the SPAREC received 14.2 mm of rain within three days of early planting and 32.5 mm of rain within two days of late planting. The Goldman Farm delayed early and late planting by 1-2 weeks (TABLE 4-1) and did not receive any rain until the eighth-day post-planting when it received 32.5 mm of rain. For late planting, the Goldman Farm received 37.1 mm of rain on the third- and fourth-day post-planting. Late mungbeans at the Goldman Farm received the benefit of more rainfall after sowing than early mungbeans, but they were planted in more clayey-loam soil rather than the more advantageous sandy loam of the early mungbeans (FIGURE 4-2).

TABLE 4-2 Analysis of Variance of the effects of planting date, cultivar, and location on yield and yield components in 2021.

Source	**df**	**Yield**	**Plant Ht.**	**Pods · Plant^{-1}**	**Seeds · Pod^{-1}**	**Seed Size**
		Pr > f				
Southern Piedmont AREC						
Rep	3	0.01**	ns	ns	ns	ns
Plant Date	1	ns	0.02*	ns	ns	ns
Rep*Plant Date [a]	3	-	-	-	-	-
Cultivar	1	ns	0.03*	ns	ns	ns
Rep*Cultivar [b]	3	-	-	-	-	-
Plant Date*Cultivar	1	0.04*	ns	ns	ns	ns
CV (%)		5.27	10.31	16.52	3.06	7.92
Brick Goldman Farm						
Rep	3	ns	ns	ns	ns	ns
Plant Date	1	ns	ns	ns	ns	0.02*
Rep*Plant Date [a]	3	-	-	-	-	-
Cultivar	1	ns	ns	ns	ns	ns
Rep*Cultivar [b]	3	-	-	-	-	-
Plant Date*Cultivar	1	ns	ns	ns	ns	ns
CV (%)		15.43	3.39	28.29	3.42	17.41

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] used as an error term for Plant Date ANOVA

[b] used as an error term for Cultivar

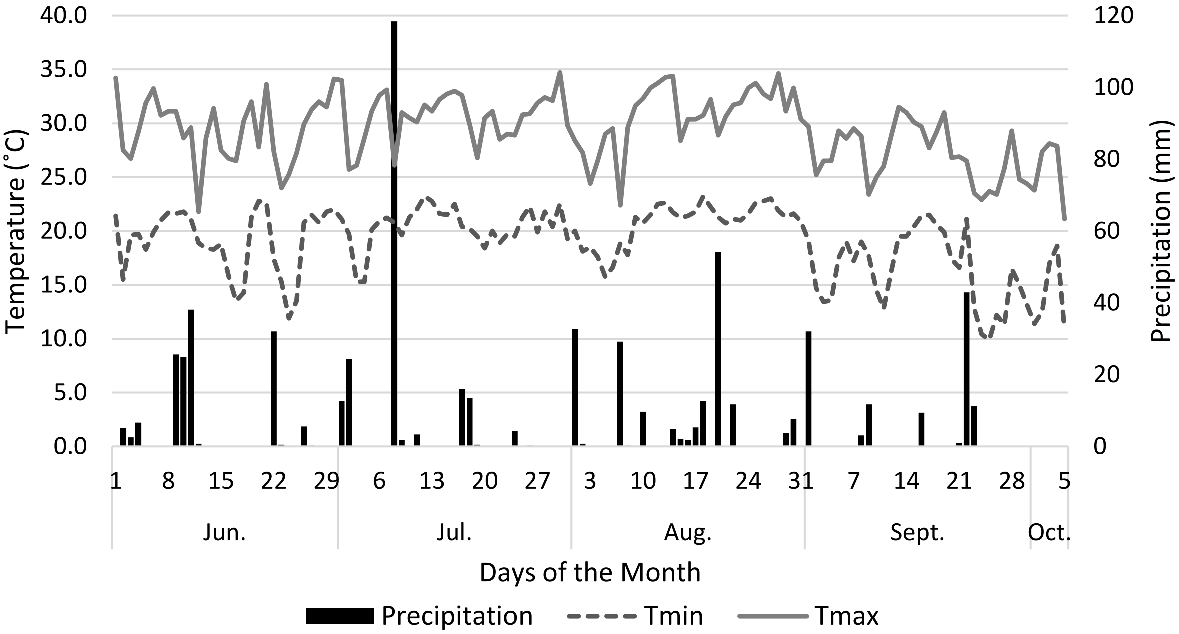

FIGURE 4-2 Climatograph based on weather reports from June 1 to October 5, 2021 at Southern Piedmont AREC.

At Red Handle Farm, the yield ranged from 0.19 (Berken in late planting) to 0.54 MT ha^{-1} (Berken in early planting) with an average of 0.19 MT ha^{-1} (FIGURE 4-3). Yield at the early planting date was significantly higher than the late planting date, similarly to SPAREC, early Berken yielded twice as much as late Berken, while OK 2000 yielded 0.05 MT ha^{-1} more in early planting than late planting (TABLE 4-2). There were no significant differences between cultivars for yield.

In 2022, early and late planting for the SPAREC and Red Handle Farm were sown on the same dates. Within the first week of early planting, the fields received 19.6 mm of rain, though a majority of the rainfall was on days five and six post-planting. In late planting, the fields received less than 2.5 mm of rain in the first week and less than 22.1 mm in the proceeding twenty-four days. At the SPAREC, due to dry conditions, both planting dates were irrigated with

25.4 mm of water after sowing but received no more irrigation after that through the growing season. Red Handle Farm was solely rain-fed and did not receive any irrigating after sowing.

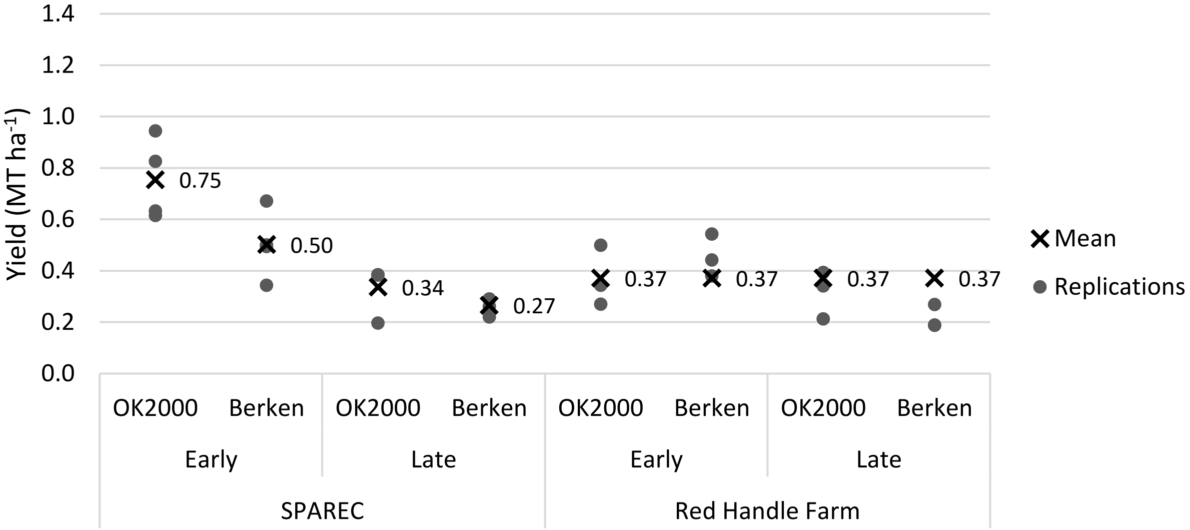

FIGURE 4-3 Spread and means of yield data from all replications of OK 2000 and Berken in early and late plantings at two locations in 2022.

Through observation, it was clear that the late planting date for both locations in 2022, particularly at Red Handle Farm, appeared stunted for a majority of the growing season and only bulked near the last two weeks before harvest. In addition to drier conditions, both locations had higher weed pressure on the later planting dates, due to less maintenance. This can explain the lower yields at both locations on the later planting dates. The higher overall yields at SPAREC compared to Red Handle Farm is likely a response to SPAREC irrigation post-planting as well as the longer growing season. SPAREC plots were harvested 1-2 weeks later than Red Handle Farm due to labor availability and resources.

In 2021 and 2022, according to the weather data recorded at the SPAREC, while temperatures were mostly consistent between years, precipitation varied greatly. The average temperature from June to September was 23.7˚C with a range of 9.9˚C to 34.7˚C in 2021 (FIGURE 4-2) and an average of 23.7˚C with a range of 6.6˚C to 37.2˚C in 2022 (FIGURE 4-4). Meanwhile, rainfall from June to September in 2021 (FIGURE 4-2) was 193 mm higher than in 2022 (FIGURE 4-4) with 625 mm and 431.5 mm, respectively. Though precipitation was much higher in 2021, it was consistent throughout the summer with only one dry spell of 10 days with less than 12.7 mm total of rain in mid-June and one tropical storm Elsa with 118.0 mm on July 8. There were nine heavy rains above 25.4 mm and only three days with more than 38.1 mm. Rainfall in 2022, however, was very scarce with a cluster of heavy rain in mid-summer. June went twenty-one continuous days with less than 12.7 mm of rain and less than 25 mm of rain in the whole month.

TABLE 4-3 Analysis of Variance of the effects of planting date, cultivar, and location on yield and yield components in 2022.

Source	**df**	**Yield**	**Plant Ht.**	**Pods · Plant^{-1}**	**Seeds · Pod^{-1}**	**Seed Size**
		Pr > f				
<u>Southern Piedmont AREC</u>						
Rep	3	ns	ns	ns	ns	ns
Plant Date	1	0.02*	0.006**	ns	ns	ns
Rep*Plant Date [a]	3	-	-	-	-	-
Cultivar	1	0.03*	ns	ns	ns	ns
Rep*Cultivar [b]	3	-	-	-	-	-
Plant Date*Cultivar	1	ns	ns	ns	ns	ns
CV (%)		28.90	8.86	24.19	7.03	13.00
<u>Red Handle Farm</u>						
Rep	2	ns	ns	ns	ns	ns
Plant Date	1	0.05*	ns	ns	ns	ns
Rep*Plant Date [a]	2	-	-	-	-	-
Cultivar	1	ns	ns	ns	ns	ns
Rep*Cultivar [b]	2	-	-	-	-	-
Plant Date*Cultivar	1	ns	ns	ns	ns	ns
CV (%)		15.19	10.62	44.75	9.77	11.65

*Significant at the .05 probability level. **Significant at the .01 level. Ns, nonsignificant at the .05 probability level.

[a] used as an error term for Plant Date ANOVA

[b] used as an error term for Cultivar

From July 25 to August 15, there was a total of 302.8 mm of precipitation. This three-week period accounted for 70% of the entire summer's rainfall. The drastic environmental differences in rainfall between years likely cause the large decrease in yield from 0.83 MT ha^{-1} in 2021 to 0.41 MT ha^{-1} in 2022.

Plant Height

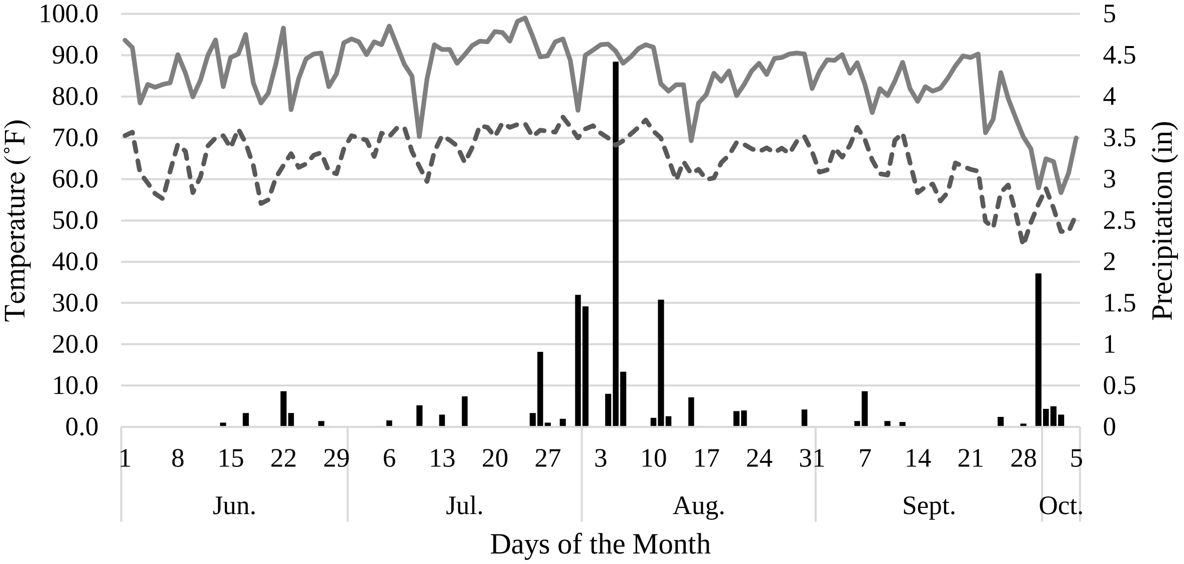

FIGURE 4-4 Climatograph based on weather reports from June 1 to October 5 at SPAREC in 2022.

There were significant differences between planting date and between cultivars for plant height at SPAREC in 2021. Early planted mungbean plants were significantly taller than late planted mungbean plants and Berken was significantly taller than OK 2000 (TABLE 4-2). Berken had the same average planting height for both planting dates, but OK 2000 was an average of 3.0 cm taller in early planting than late planting (TABLE 4-4). Plant height was also significantly different between planting dates at SPAREC in 2022 (TABLE 4-3). Early plantings were an average of at least 9.0 cm taller than late plantings. This was likely due to higher weed pressure and drought conditions followed by extreme water during the early vegetative growth stages in the late planted beans. No significant differences were observed for plant height between planting dates or between cultivars at the Goldman Farm in 2021 and the Red Handle Farm in 2022.

Pods per Plant

There were no significant differences between planting dates or between cultivars for the number of pods per plant for any of the locations or years, however, some locations had noticeably more pods per plant (TABLE 4-2) (TABLE 4-3). Since no statistical analysis was run across locations or by year, it can only be noted which locations had higher pod counts. The SPAREC had an average pod count of 51.25 pods per plant in 2021 and 88 pods per plant in 2022. The Goldman Farm in 2021 had an average pod count of 67.25 and Red Handle Farm in 2022 had an average pod count of 61 pods per plant (TABLE 4-4).

Seeds per Pod

Number of seeds per pod was not significantly different between planting dates or between cultivars in any of the locations or years (TABLE 4-2) (TABLE 4-3). In 2021, the SPAREC and the Goldman Farm had averages of 10.75 and 10.45 seeds pod^{-1}. The number of seeds per pod ranged from 9.6 to 11.5 in 2021 with an average of 10.6 seeds pod^{-1} across both locations. In 2022, the SPAREC and Red Handle Farm had averages of 12.1 and 11.9 seeds pod^{-1}. The minimum number of seeds pod^{-1} was 10.7 while the maximum was 14.2 and the average was 12.1 seeds pod^{-1} (TABLE 4-4).

Seed Size

There were no significant differences in seed size between planting dates and between cultivars at SPAREC in 2021 and 2022 at the Red Handle Farm. Seed size was significantly different between planting dates at the Goldman Farm in 2021. Seeds from the early planting were significantly larger than seeds from the later planting. (TABLE 4-2) (TABLE 4-3). There were no significant differences between cultivars for seed size at the Goldman Farm in 2021. The seed size in 2021 at the SPAREC, however, was smaller with an average of 3.9 g 100 seeds^{-1} while in 2022 it was 5.2 g 100 seeds^{-1} at the SPAREC, 5.3 g 100 seeds^{-1} at the Goldman Farm, and 4.7 g 100 seeds^{-1} at Red Handle Farm (TABLE 4-4). Seed size is correlated to genetics and the quality of the seed. Since the seed size for Berken and OK 2000 is genetically similar, we can use seed size in these experiments as a measure of seed quality. The SPAREC in 2021 was harvested later than any of the other locations or years and it was observed that the seed quality was much worse. The seeds had more mold and wrinkled seeds than other fields which correlates to its smaller seed size.

TABLE 4-4 Average yield components of Berken and OK 2000 in all locations from 2021-2022.

Planting Date	Cultivar	Plant Ht.	Pods · Plant^{-1}	Seeds · Pod^{-1}	Seed Size
		cm			g · 100 seed^{-1}
			2021		
SPAREC					
Early	Berken	44	54	10.7	3.4
Early	OK 2000	36	51	10.4	4.0
Late	Berken	44	55	10.9	3.7
Late	OK 2000	33	45	11.0	4.5
Brick Goldman Farm					
Early	Berken	28	88	10.4	5.4
Early	OK 2000	24	64	10.5	5.6
Late	Berken	28	57	10.5	5.3
Late	OK 2000	29	60	10.4	4.9
			2022		
SPAREC					
Early	Berken	42	99	13.2	4.8
Early	OK 2000	34	103	11.7	5.4
Late	Berken	32	69	12.4	5.1
Late	OK 2000	28	81	12.0	5.6
Red Handle Farm					
Early	Berken	40	48	12.2	4.4
Early	OK 2000	39	48	11.6	4.2
Late	Berken	32	65	11.6	5.0
Late	OK 2000	32	83	12.0	5.2

Conclusion

There is great potential for mungbean as an alternative crop for southern Virginia tobacco farmers. In some cases, yields reached as high as 1.18 MT ha^{-1} which is higher than global average of 1.13 MT ha^{-1} (Chauhan & Williams, 2018; Thomas et al., 2004). In other cases, the lowest yield was 0.19 MT ha^{-1} and the average yield for 2021 was 0.84 MT ha^{-1} and for 2022 was 0.38 MT ha^{-1}. In India, the highest-producing country of mungbean, the average yield is 0.5 MT ha^{-1} (Nair & Schreinemachers, 2020). A similar field trial for production of mungbean in piedmont Virginia found yields ranging from 1.15-1.47 MT ha^{-1} (Bhardwaj & Hamama, 2015).

Planting date was a significant effect on yield in 2022 and on plant height at SPAREC in both years. This may, however, be due to less precipitation and weed management in later planting. Proper irrigation and herbicide use could allow for a wider planting date range. The interaction of plant date and cultivar was significant at SPAREC in 2021 with OK 2000 performing better in late planting and Berken performing better in early planting.

In addition to variability in yield and yield components, obstacles to Virginia farmers producing mungbean include a lack of a supply chain and problems with mechanical harvesting. Though mungbeans are able to be combined similarly to soybean, there needs to be more research on when to spray the desiccant so that the plant is dry enough to be combined without decreasing seed quality. Berken and OK 2000 varieties have extreme lodging problems in Virginia which make them unable to be mechanically harvested. Lastly, a difficulty to mungbean in Virginia is the indeterminant growth habit of available cultivars. This makes it hard to decide the optimal harvest time since the pods are mature in stages, which brings the urgent need of developing determinant cultivars.

Though there are many challenges, the highest yields show evidence of the potential success of mungbean in Virginia. Many of the current issues can be solved through proper production management and breeding efforts. Extension specialists in agronomy, plant pathology, entomology, soil nutrient, etc. could be involved in establishing a production guide. For example, mungbean fits well with common Virginia crop rotations with winter wheat but should be treated as more of a vegetable crop with more attentive management and higher profit. Breeding programs could utilize publicly available germplasm from the World Vegetable Center who maintains 6,700 mungbean accessions (Schafleitner et al., 2015). All the efforts will receive a significant economic return as Myanmar received an estimated return on investment of 92 USD in return for every dollar spent on breeding efforts beginning in 1980 (Sequeros et al., 2020). The investment in mungbean development for southern Virginia has the potential to have large economic returns and be advantageous as an alternative crop for tobacco farmers.

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Chapter 5. Exploring Nutritional Composition and Volatile Compounds of Soybean, Edamame, and Mungbean

Exploring Nutritionala Composition and Volatile Compounds of Soybean, Edamame, and Mungbean

Jessica Wilbur

ABSTRACT

As the global population grows, animal product demand is forecasted to increase by 50-70% by 2050 and is a major source of greenhouse gas emissions. This has resulted in a bigger consumer demand for plant-based proteins including soyfoods, vegetable soybean and edamame. There is also an increase in plant-based meat and egg alternatives often made with mungbean isolate proteins. The long-term goal of our project is to explore suitable beans for plant-based protein products. The objective of this study is to investigate the nutritional composition and volatile profile of soybean, edamame, and mungbean. We used two commercial cultivars for each bean (soybean: MFS 995 and USG 5618V, edamame: VT Sweet and UA Kirksey, and mungbean: Berken and OK 2000). Crude protein was quantified using the Kjeldahl method, crude fat was measured using the Soxhlet Ether Extraction Method, ash was determined by the dry ashing method, and NDF was analyzed using the ANKOM^{2000} Fiber Analyzer method. The data was analyzed using JMP to run a One-Way ANOVA. Volatile analysis was analyzed by gas chromatography-mass spectrometry (GC-MS). Results showed that, nutritionally, edamame cultivars had the highest protein content with an average of 38.30% crude protein in dry solid, followed by soybean with 36.17%, and then mungbean with the lowest crude protein content at 21.12%. The edamame and soybean cultivars had significantly higher crude fat content with an average of 14.00% and 13.47% respectively in dry solid, than the mungbean cultivars which had 0.77% crude fat. Volatile profiles were similar between cultivars of the same bean but varied dramatically among crops. Acetic acid and 2-butanone were most notably higher in soybean,

which contributes to a vinegary note. Hexanal, which has a tallowy, leaf-like aroma, was equally present in soybean and mungbean but lower in edamame. Edamame was highest in 1-octen-3-ol with a mushroom-like aroma and was the only bean containing octanal with a fatty odor, and nonanal with a fruity aroma. Mungbean had the highest 1-hexanol content with a floral note. The diverse nutritional and volatile profiles of each bean suggested potentially unique applications in foods, though the interaction of the aromas and other quality attributes in each bean needs to be studied. This information can be used as a preliminary guide for producing soybean-, edamame-, or mungbean-based food products.

Introduction

Currently, 83% of global farmland is used to produce animal products that only provide 18% of calories to the global human diet (Poore & Nemecek, 2018). With the projected population increase in the coming years, meat demands could increase land needs for livestock by 204%. Meanwhile, substituting 50% of animal products with plant proteins could reduce needed land by 35% (Alexander et al., 2017). More recently in the western world, there has been a greater push towards more "sustainable" diets containing fewer animal products and more plant protein. Terms such as "flexitarian," or a "person who sometimes eats meat or fish although they do not usually do so," (Oxford English Dictionary, 2022) are emerging in addition to vegetarians, pescatarians, and vegans. Campaigns in Germany and the Netherlands like "less but better" refers to consuming smaller portions of meat balanced with plant proteins or "meatless days" which encourages going full days without eating meat (de Boer et al., 2014). Food technology is revolutionizing plant protein options through meat alternatives to support these plant focused diets (Alexander et al., 2017; Bonny et al., 2015).

Plant proteins from legumes are appealing for more than just their sustainability. They have added health benefits of isoflavones, antioxidants, minerals, amino acids, vitamins and low cholesterol, which can reduce the risk of diabetes, cancer, hypertension, and other diseases (Bessada et al., 2019; Boye et al., 2010; Carlos et al., 2017; Hertzler et al., 2020; Hou et al., 2019; Keatinge et al., 2011). In terms of producing meat alternatives, legume crops are high in protein and have important emulsification properties which add to the texture and mouthfeel of food (Kim et al., 2020).

Soybeans are the most produced legume crop in the world with a global production of 390,526,000 MT in 2022 (USDA, 2022b). Soybean requires processing before eating and can be made into a variety of traditional foods such as soymilk, tofu, tempeh, and natto. The production and versatility have led soybean to be one of the most commonly used plant proteins for meat alternatives (Santo et al., 2020).

Edamame is a vegetable soybean that is harvested while the pods are still immature. It is typically boiled and salted and then consumed directly out of the pod. Edamame is not normally processed and used for protein in alternative meat products. However, it can be shelled and consumed as a high protein food option or added to meals and dishes. In the United States, edamame demand is estimated to be increasing by 12 to 15% every year (Reiter et al., 2019).

Mungbean is a pulse crop that can be used in traditional Asian and South Asian cuisine such as dahl or as a bean sprout (Lambrides & Godwin, 2007). Demand for mungbean is currently growing due to its potential for use in alternative animal products. Companies such as BeyondBurger (Beyond Beef) and JUST Egg (JUST Egg) use mungbean as a primary ingredient in their plant-based burger patties and plant-based egg alternatives, respectively. The growing use of mungbean in plant-alternative proteins is reflected by the increase in imports of mungbean to the United States which increased by to 111% from the 2018/2019 to 2020/2021 market year (USDA, 2022a).

When it comes to plant proteins, overall health benefits of the product and flavor are the biggest decision-making factors for consumer acceptance. More often than not, flavor in these products was a previous deterrent to consumers purchasing plant proteins (Onwezen et al., 2021). The first objective of this study is to find and compare nutritional composition (crude protein, crude fat, Neutral detergent fiber (NDF), and ash) of two cultivars of soybean, edamame, and mungbean. The second objective is to investigate aroma profiles of three bean cultivars. This work is not only important in breeding goals for selecting beans with more desirable nutrition and aroma attributes, but also important for further understanding the use of soybean, edamame, and mungbean in alternative protein products.

Materials and Methods

Sample Materials

Two cultivars of soybean (USG 5618V and MFS 995), two cultivars of edamame (UA Kirksey and VT Sweet), and two cultivars of mungbean (Berken and OK 2000) were used for this study. Soybeans were grown in 2021 and edamame in 2019 at Kentland Farm in Blacksburg, VA while mungbean seeds were purchased from the Oklahoma Seed Foundation (Stillwater, OK 74075) in 2021. Mungbean and soybean seeds were stored in the same, dry storage room in Blacksburg, VA for a year prior to the study at a seed moisture of 9 to 11%. Edamame cultivars used for nutritional analysis were grown as soybean, harvested at full maturity, and then stored in the same storage room as soybean and mungbean. Edamame used for volatile analysis was harvested as a vegetable crop and immediately shelled prior to being stored in a 0˚C freezer.

Nutritional Methods

To prepare soybean, edamame, and mungbean samples, dry seeds were ground using a Wiley mill to grind into a fine powder for all nutrient analysis. To determine crude protein, the Kjeldahl method was performed using Foss Tecator^{TM} 20-tube digester (Foss Analytical AB, Höganäs, Sweden). This method measures nitrogen content which is then used to calculate crude protein by multiplying N by 6.25. Crude fat was found using a Soxtec extraction unit Model HT6 Foss (North America, Inc., Eden Prairie, MN 55344) and Soxhıet ether extraction method. The ANKOM 2000 Fiber analyzer and NDF method were used for measuring NDF. And ash was determined using a muffle furnace at 550˚C for 12 hours (Jin et al., 2019). All nutritional factors were determined in solid dry weight.

Volatile Methods

To prepare mungbean and soybean samples, 15.0 g of dry seeds and 10.0 g of dry ice were ground to free-flowing powders in bean-specific coffee grinders. Once the dry ice was fully sublimated, 2.0 g of the ground seed sample was weighed into a clean, clear glass, screw cap, 20ml vial (Supelco, Bellefonte, PA) and flushed with nitrogen. Edamame samples were prepared in a similar way except frozen edamame was ground with dry ice and the samples were weighed into the vials in a nitrogen-flushed glovebox to prevent interference of volatiles through humidity or oxygen exposure. All samples were analyzed in triplicate.

3-hexanol (Stableflex, Supelco, Bellefonte, PA) was used as the internal standard for each bean because it had no overlap in the retention time of other detected volatile compounds. HPLC grade Methanol (Stableflex, Supelco, Bellefonte, PA) was used to dilute the standard specifically to each bean so that the peak area would be comparable in scale to the peak areas of that bean's volatile compounds. The internal standard for edamame was 1.0 μL of 10^{-2} diluted 3-hexanol and for soybean and mungbean, the internal standard was 2.0 μL of 10^{-4} diluted 3-hexanol.

Headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) with Agilent Gas Chromatography 6890N equipped with 5975B Mass Spectrum Detector and Leap Technologies CTC PAL system autosampler was used for volatile compound characterization. For extraction, 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibers (Stableflex, Supelco, Bellefonte, PA) were used for each bean type. Volatile incubation was performed at 45˚C for 30 minutes. The GC injection was completed in hot splitless mode for all three beans. The initial oven temperature of 40˚C was held for 5 minutes, and then increased by 5˚C per minute for 20 minutes until a temperature of 225˚C was reached and held for another 20 minutes.

Major volatile compounds were identified through a NIST library and by comparing present literature to detectable peaks in each bean. Compound identification was performed through the calculation of the retention index of each compound against C7-C30 alkane according to the method developed by Van den Dool and Kratz (1963). The abundance of those compounds was measured by the peak area ratio of targeted compounds to the corresponding internal standard on MS chromatogram. The obtained ratio for some shared volatile compounds allowed for comparison across beans.

JMP Pro 16 was used to analyze a one-way ANOVA for both nutritional data and volatile data. Cultivar was used as the main effect and a Tukey's HSD test was performed with alpha ($p<0.05$).

Results

Part I. Nutrition Results

Crude Protein

Edamame was highest in crude protein ranging from 37.7- 38.8%, while soybean ranged in protein from 35.5- 36.5%, and mungbean had the lowest crude protein with a range from 20.3-22.1%. UA Kirksey, with an average of 38.7% protein, was significantly higher than all cultivars of mungbean and soybean, while VT Sweet, with an average of 37.9% protein, was statistically similar to USG 5617V (

TABLE 5-1). MFS 995 and USG 5618V soy had averages of 36.0% and 36.4% protein, respectively, which were both significantly higher in crude protein than Berken and OK 2000 mungbeans with averages of 20.6% and 21.7%, respectively (

TABLE 5-1).

Crude Fat

Crude fat was not significantly different amongst edamame and soybean cultivars from 12.8% in MFS 995 soy and 14.5% fat in VT Sweet edamame. Edamame and soy were significantly higher than both mungbean cultivars which only had 0.646- 0.934% lipid content. The average fat for both cultivars of soybean was 13.5%, edamame was 14.0%, and mungbean was 0.769% (

TABLE 5-1).

Neutral Detergent Fiber

NDF was lowest in Berken mungbean at 9.4% and highest in MFS 995 soy at 15.9% fiber. Berken and MFS 995 were significantly different from each other but not from the other cultivars of all three beans. The remaining cultivars ranged from 9.5% in OK 2000 mungbean to 13.8% fiber in USG 5618V soy. The average NDF for both cultivars of soybean was 14.7%, edamame was 11.0%, and mungbean was 10.6% (

TABLE 5-1).

Ash

Ash was highly variable across beans and cultivars. UA Kirksey edamame had the highest ash content at an average of 5.6% which was statistically similar to MFS 995 soy at 5.4% ash. Third highest in ash was USG 5618V soy at 5.3% which was statistically similar to MFS 995, but not UA Kirksey. VT Sweet edamame at 4.9% ash, followed by Berken mungbean at 3.5% ash, and then OK 2000 mungbean at 3.8% ash were each significantly lower than each other (

TABLE 5-1).

TABLE 5-1 Average proximate nutritional data in dry weight for soybean, edamame, and mungbean.

Nutritional Component	**Soybean**		**Edamame**		**Mungbean**	
	MFS 995	**USG 5618V**	**UA Kirksey**	**VT Sweet**	**Berken**	**OK 2000**
	% in dry weight ±SE					
Crude Protein	35.991 ±0.654c	36.357 ±0.103bc	38.718 ±0.104a	37.891 ±0.236ab	20.571 ±0.434d	21.670 ±0.584d
Crude Fat	13.008 ±0.308a	13.927 ±0.412a	13.869 ±0.252a	14.134 ±0.493a	0.879 ±0.083b	0.659 ±0.018b
NDF	15.706 ±0.242a	13.610 ±0.237ab	10.690 ±1.448ab	11.271 ±0.200ab	9.643 ±0.330b	11.551 ±2.866ab
Ash	5.434 ±0.026ab	5.308 ±0.056b	5.552 ±0.128a	4.935 ±0.035c	3.541 ±0.015e	3.783 ±0.006d

Note. Within rows, means followed by the same letter are not significantly different according to HSD (.05).

Part II. Volatile Results

TABLE 5-2 Relative abundance of selected volatile compounds and aroma descriptors for soybean, edamame, and soybean.

		Soybean		Edamame		Mungbean	
Volatile Compound	**Odor Descriptors**	**MFS 995**	**USG 5618V**	**UA Kirksey**	**VT Sweet**	**Berken**	**OK 2000**
		Mean Compound peak area: IS peak area ±SE					
2-Butanone	Etheric (Rychlik et al., 1998)	0.906 ±0.110a	0.775 ±0.595a	ND	ND	ND	ND
Hexanal	Tallowy, leaf-like (Rychlik et al., 1998)	1.455 ±0.135a	1.221 ±0.020a	0.158 ±0.018b	0.264 ±0.044b	1.610 ±0.271a	1.226 0.186a
1-Pentanol	Balsamic (Acree & Arn, 2004)	0.164 ±0.014ab	0.149 ±0.011b	0.136 ±0.019b	0.139 ±0.001b	0.158 ±0.021ab	0.199 ±0.024a
Octanal	Fatty (Rychlik et al., 1998)	ND	ND	0.022 ±0.001a	0.024 ±0.003a	ND	ND
1-Hexanol	Green, flowery (Rychlik et al., 1998)	ND	ND	0.004 ±0.001b	0.009 ±0.001b	0.287 ±0.067a	0.373 ±0.041a
Nonanal	Tallowy, fruity (Rychlik et al., 1998)	ND	ND	0.006 ±0.000a	0.007 ±0.001a	ND	ND
Acetic acid	Vinegar-like, pungent (Rychlik et al., 1998)	2.066 ±0.254a	2.261 ±0.160a	ND	ND	0.479 ±0.107b	0.818 ±0.142b
1-octen-3-ol	Mushroom-like (Rychlik et al., 1998)	0.148 ±0.020bc	0.176 ±0.042b	0.181 ±0.022b	0.343 ±0.023a	0.077 ±0.012d	0.104 ±0.023cd

Note. Figures were peak area ratio of selected volatile compounds to internal standards. Within rows means followed by the same letter are not significantly different according to HSD (.05).

Alcohols

1-Pentanol existed in all six varieties from the three beans. Its abundance in edamame seemed to be the lowest compared to either soybean or mungbean. Mungbean's peak ratio in 1-pentanol has a minimum of 0.134 in Berken and a maximum of 0.220 in OK 2000 with an overall average of 0.179. Soybean starts at a 0.137 ratio of 1-pentanol in MFS 995 to 0.175 in USG 5618V and averages at 0.157. Edamame ranges from 0.116 in UA Kirksey to 0.152 in VT Sweet and has an average of 0.138 1-pentanol.

1-Hexanol was higher in mungbean than edamame and not present in soybean. Mungbean 1-hexanol peak ratio ranged from 0.210 in Berken to 0.413 in OK 2000 with an average of 0.330. Edamame ranged from 2.73 x 10^{-3} 1-hexanol in UA Kirksey to 9.68 x 10^{-3} in VT Sweet and had a mean of 6.34 x 10^{-3}.

1-Octen-3-ol was present in all three beans but significance varied between cultivars of edamame. 1-octen-3-ol was significantly highest in VT sweet with an average of 0.343 and lower in UA Kirksey at an average of 0.181. Both soybean cultivars were statistically similar to UA Kirksey ranging from 0.128 in USG 5618V to 0.201 in MFS 995 and averaging at 0.162. 1-Octen-3-ol in OK 2000 was similar to MFS 995 with a ratio average of 0.104 and Berken was similar to OK 2000, but not MFS 995 with an average of 0.077 1-octen-3-ol. Mungbean overall ranged from 0.065 in Berken to 0.129 in OK 2000 and had a mean of 0.0906.

Ketone

2-Butanone was only present in soybean and ranged from a peak ratio of 0.817 in USG 5618V to 1.18 in MFS 995 and a combined average of 0.979.

Aldehydes

Hexanal was significantly highest in soybean and mungbean ranging from a peak ratio of 1.07 in OK 2000 to 1.83 in Berken with an average of 1.42 in mungbean and 1.34 in soybean. Hexanal was present in edamame in a smaller amount ranging from 0.139 in UA Kirksey to 0.313 in VT Sweet and a mean of 0.211.

Octanal was only present in edamame and ranged from 0.0208 in UA Kirksey to 0.0275 in VT Sweet. The average peak ratio of Octanal in edamame was 0.0227.

Nonanal was only detected in edamame which ranged from 5.70×10^{-3} in UA Kirksey to 8.07×10^{-3} in VT Sweet. The average peak ratio of nonanal in edamame was 6.25×10^{-3}.

Carboxylic Acid

Acetic acid was higher in soybean, lower in mungbean, and undetected in edamame. For soybean, acetic acid was present at a ratio minimum of 1.79 in USG 5618V and maximum of 2.40 in MFS 995 and an average of 2.16. In mungbean, acetic acid was lowest in Berken at 0.360 and highest in OK2000 at 0.981. The average of acetic acid in mungbean was 0.649.

Discussion and Conclusion

The overall findings showed that edamame is the highest in crude protein with an average of 38.3%, followed by soybean with 36.1% crude protein, and mungbean had the lowest crude protein at 21.1%. The significantly lower protein content in mungbean can be a downside when compared to soybean or edamame but is still considered to be high in overall protein.

Crude Fat was similar in soybean and edamame at 13.7% but much lower in mungbean at only 0.77% fat. Depending on what the vegetable protein is used for, higher fat can be beneficial for emulsification and whipping capacities as well as for use as binders (Thompson et al., 1982). Soyfoods, including edamame, is a major source of essential fatty acids (Tripathi & Shrivastava, 2017). Contrarily, in terms of flavor, oxidation of unsaturated fatty esters can contribute to the off-flavor referred to as a “beany flavor” that can be undesirable to consumers in western cultures, but desired in some Asian diets (Rackis et al., 1979; Shahidi & Hossain, 2022). Removal of lipids for food processing can result in a change of other functional properties such as color which can negatively affect consumer perception (Mehle et al., 2020). This could give mungbeans, with a much lower lipid content, an advantage for alternative meats if the goal is to remove “beany” flavor.

NDF was similar across all beans at an average of 12.1% fiber, with only MFS 995, the highest in fiber, and Berken, the lowest in fiber differing significantly. NDF can give us relative information about dietary fiber but is not a true estimate of fiber in human diet. High fiber is extremely beneficial to gut microbiome and overall digestion and is much higher in vegetable-based diets (Mehle et al., 2020).

Ash is important because it indicates the overall mineral content within a food product, though doesn’t specify which minerals. Ash was variable amongst all beans and cultivars with a range of 3.78- 5.56% ash. This gives room for potential breeding efforts to increase overall mineral content since even within cultivars of the same bean, there were significant differences. Further analysis on which minerals are present or most abundant could help further understand nutritional benefits of each bean.

In addition to health aspects, the number one driver, or in most cases a deterrent, for consumers was the aroma of plant proteins (Onwezen et al., 2021). In western cultures, the biggest off-flavor for legume plant proteins is their “beany” flavor (Fischer et al., 2022). Contrarily in Asian cuisine, beany flavor may be more desirable as palatability is variable between cultures (Keast & Lau, 2006) The “beany” flavor can be altered through different processing techniques such as temperature treatments (including blanching, freezing, pasteurizing, roasting), grinding or milling, fermentation, drying, and texturizing (Fischer et al., 2022; Mital & Steinkraus, 1979; Roland et al., 2017; Trindler et al., 2022). Breeding efforts, cultivars, on-farm practices, harvest, and storage practices can also alter “beany” flavors (Mehle et al., 2020; Shahidi & Hossain, 2022; Trindler et al., 2022).

“Beany” flavor has been identified through association with certain volatile compounds such as 1-hexanol, nonanal, and 1-octen-3-ol (Bott & Chambers Iv, 2006; Trindler et al., 2022). Additionally, though hexanal does not have a “beany” aroma independently, in combination with other “beany” volatiles, it can greatly enhance the “beany” flavor.

In this study, it was found that the soybean, edamame, and mungbean have unique aroma profiles, while a majority of the main volatile compounds in each contribute to overall “beany” flavor. It was also found that between cultivars of the same bean, major volatiles were not significantly different, except for 1-octen-3-ol in edamame (TABLE 5-2). Nonanal was only detected in edamame, though other studies have found the presence of nonanal in both soybean and edamame (Boué et al., 2003; Han et al., 2022). 1-octen-3-ol was higher in edamame than soy and mungbean only when considering both cultivars together, but VT sweet was significantly higher in 1-octen-3-ol than UA Kirksey and edamame was much lower in hexanal. Meanwhile, soy and mungbean were significantly higher in hexanal and mungbean was higher in 1-hexanol.

Other non-“beany” volatiles were detected in soybean such as 2-butanone that wasn’t present in edamame or mungbean, and acetic acid that was detected in a lesser amount in mungbean and not at all in edamame. 2-Butanone has an etheric aroma resembling grape skins or wine while acetic acid can resemble a more pungent or vinegar-y aroma (Rychlik et al., 1998). 1-Pentanol which has a sweeter, balsamic aroma (Acree & Arn, 2004) was present in all three beans but was highest in mungbean.

The nutritional profiles and volatile profiles measured in this study can be used in the future for a better understanding of how soybean, edamame, and mungbean compare when deciding on food production and processing of alternative plant proteins. It also can be used by breeders to show which aspects of each bean are more desirable and less desirable to consumers.

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