Evaluation of Selected Edamame Cultivars for Isoflavone Content, Organoleptic Characteristics, and Production in Central Alabama

by

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Abstract

Edamame [Glycine max (L.) Merr.] is vegetable soybean harvested and consumed at the R6 development stage. The growing popularity of edamame as a healthy snack food has led to increased interest in edamame production from soybean producers across the southeast. Little is known about edamame production methods or the commercially available cultivars currently on the market. As a result, four studies were conducted to evaluate edamame cultivars both in terms of production as well as isoflavone content and organoleptic characteristics. Thus the objectives of these studies were to 1) evaluate selected commercially available edamame cultivars for use in large scale production; 2) determine the isoflavone content of each of the cultivars evaluated for production in the southeast; 3) evaluate consumer preference and the sensory attributes of selected cultivars that showed promise for commercial production; and 4) evaluate the response of selected edamame cultivars to incremental increases in potassium fertilizer rates. Data collected in these studies resulted in new understanding of selected cultivars to include yield and yield components, isoflavone content and distribution, cultivar preference by consumers, and potassium fertility recommendations. The results of the four studies provide growers, retailers, consumers, and packagers with useful, previously unavailable data on many of the most widely available commercial edamame cultivars on the market in the United States. Using this data, interested parties can make production decisions based on current information covering many aspects of edamame and its production.

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List of Abbreviations

MG	Maturity group
AVSE	American Vegetable Soybean and Edamame
P	Phosphorus
K	Potassium
N	Nitrogen
RCBD	Randomized complete block design
UHPLC	Ultra-high performance liquid chromatograph

I. Edamame [*Glycine max* (L.) Merr.] Cultivar Evaluation in Central Alabama

Abstract

Edamame [*Glycine max* (L.) Merr.] is vegetable soybean harvested and consumed at the R6 development stage. The growing popularity of edamame as a healthy snack food has led to increased interest in edamame production from soybean producers across the southeast. The objective of this study was to evaluate selected edamame cultivars for adaptation and production in central Alabama. Selected cultivars were represented by four maturity groups : MG III (Midori Giant, Chiba Green, Butterbean, Sayamusume, and BeSweet 2001), MG IV (Gardensoy 42, Mojo Green), MG V (Mooncake, Lanco, and Gardensoy 51), and MG VI (Owens).Cultivars were planted in replicated plots at the Plant Breeding Unit in Tallassee, AL in May 2014 and June 2015. Data was collected on germination, plant height at maturity, height of lowest pod, weight of 25 shelled beans, days to first flower, pods per plant, beans per pod, and total yield. Highest yielding cultivars in 2014 included Gardensoy 51 and Midori Giant at 15,960 and 15,742 kg ha^{-1} respectively. In 2015, Mojo Green, Gardensoy 51, and Mooncake were the highest yielding cultivars at 13,576, 13,195, and 13,092 kg ha^{-1}. In both years BeSweet 2001 produced the lowest yields: 10,226 kg ha^{-1} in 2014 and 2,592 kg ha^{-1} in 2015. Weight of 25 shelled beans varied widely among cultivars. Cultivars from early maturity groups (MG's) (MG III) produced larger beans with lower overall yield. This data will help producers chose cultivars based on yield potential and market preferences.

Introduction

Edamame has a long history within East Asian culture. The first mention of soybean in Chinese text was before the 7th century BC in the *Shijing (Book of Odes)* (Shurtleff and Aoyagi, 2009). The word edamame was first documented in Japan in 1275 (Shurtleff and Aoyagi, 2009). Edamame was virtually unknown in North America until the mid-19th century. Only within the past 30 years has edamame gained widespread acceptance in the U.S (Shurtleff and Aoyagi, 2009).

In recent years, there has been an increased demand and broader acceptance of edamame in the United States. Edamame is currently the second most consumed soy food, behind soy milk, in the United States (Soyfoods, 2014). In 2013, it was estimated that the United States consumed between 22,700-27,270 tonnes of edamame (Nuss, 2013). This increased demand is expected to continue as consumers look for healthier, lower cost sources of protein to add to their diet. While edamame is an excellent source of plant based protein, it is also a complete protein, containing all the essential amino acids in the human diet (Velasquez and Bhathena, 2007). Edamame is one of only a handful of plant-based foods that contains all the essential amino acids. In addition, edamame is high in isoflavones (Wang and Murphy, 1994). Isoflavones have been reported to reduce the incidence of certain cancers, and are also powerful antioxidants (Simonne et al., 2000, Franke et al., 1994, Wang and Murphy, 1994).

In response to this increasing demand, growers, plant breeders, and food processors have grown increasingly interested in the production of edamame. Currently, 95% of edamame is imported from China into the United States (Roseboro, 2012). Based on current U.S. levels of edamame consumption and expected yields, a large increase in domestic production would be required to meet current U.S. demand. This increased demand has prompted food processers and packagers to look to local sources to fill the expanding need.

Edamame has great potential to be a profitable crop in the southeastern U.S. The University of Kentucky conducted research in the early 2000's that investigated the profit potential of edamame. It was determined that projected net returns ranged from $640 – $6,177 ha^{-1} (Ernst and Woods, 2001., Ernst, 2000). At the lower range of return, edamame is more profitable than sweet corn and pumpkins. At the high end of return, edamame can be more profitable than staked tomatoes and cantaloupe. In most cases, the breakeven price for edamame is less than $2.20 per kg, much lower than the average wholesale price of $3.30. Edamame is of particular interest to soybean farmers due to the similarities in equipment and management practices. This interest may increase in the coming years as commodity soybean prices are projected to decrease (Schnitkey, 2015). With the exception of only the highest yielding land, 2016 prices would likely not cover the costs of commodity soybean production.

Historically, growers have been somewhat reluctant to grow edamame due to the lack of processing facilities and limited production information (Binder, 2010). For edamame production to become commercially viable in Alabama, growers need adapted cultivars that will perform well in the southeastern U.S. Several studies have been conducted to evaluate edamame cultivars for both home garden and commercial production. Unfortunately, most of the studies were conducted outside of the southeastern United States (Sciarappa et al., 2007; Williams et al., 2012; Miles and Sonde, 2002). This is an important consideration due to the photoperiodic nature of soybean (Board and Settimi, 1986). Because of this, edamame cultivars from different maturity groups will bloom at different times based on the latitude in which they are grown and the subsequent day length (Cregan and Hartwig, 1984). Many cultivars imported from China are adapted to the latitude in which they were produced. Most soybean production in China falls within 41°N and 45°N latitude, while production in the southeastern U.S. falls between 30.5°N and 36°N. Based on maturity groups used in the United States, most Chinese cultivars would be MG II or MG III, best adapted to the northern U.S. Cultivars adapted to the long day lengths experienced in the northern portion of the United States will bloom prematurely under the short day lengths in the south (Cregan and Hartwig, 1984). Although premature blooming will often result in lower yields, it is unclear if lower yield will be offset by the superior size and flavor of earlier maturing cultivars common to East Asia.

While few studies have been conducted in the southeastern U.S., in most cases the results were promising. Zang and Kyei- Boahen (2007) evaluated several cultivars for production in the Mississippi Delta in 2004 and 2005. Cultivars were selected from multiple maturity groups and with differing stem termination types. Plant height was found to vary significantly among cultivars ranging from 17 to 145 cm. Beans per pod averaged around two, but data on variation in the number of beans per pod was not provided. Because Grade A edamame is required to have 90 percent or greater two and three bean pods, this data is necessary to determine the marketable portion from each cultivar (Konovsky et al., 1994). Similarly, individual bean weight was not evaluated. Although not commonly considered a major factor in commodity soybean, bean weight is an important consideration in edamame production (Konovsky et al., 1994). Last, yield data varied widely among cultivar and year, with a low of 1,605 kg ha^{-1} in 2004 and a high of 43,862 kg ha^{-1} in 2005.

Williams et al., (2012) evaluated over 100 edamame cultivars for sensitivity to commonly used herbicides in Illinois. During the trials, data were collected on emergence, plant height, and days to R1 (beginning bloom) (Fehr and Caviness, 1977). Data was not collected on yield, bean weight, or other components of yield. Emergence data varied from 0 to near 100% (Williams et al., 2012). Problems with edamame germination have been well documented (Williams et al., 2012; Hamilton, 2007). Using this data, cultivars with poor germination rates can be excluded from this evaluation in favor of high performing cultivars.

Additional studies on performance of edamame cultivars have been conducted in Arkansas, Georgia, and Virginia. Arkansas has increased edamame research since the establishment of the nearly 3000 m^2 American Vegetable Soybean and Edamame (AVSE) processing facility in 2012. Since that time AVSE has contracted with local growers to grow edamame for their production facility (Edamame Production Facts, 2013). The cultivars used are not commercially available and thus are not useful in this specific research. However, information regarding the maturity group, individual bean weight, yields, and harvest method is available. Using this information, along with data collected in Georgia (McPherson et al., 2008) and Virginia (Carson et al., 2011) it is possible to select cultivars with the highest potential for success for southeastern production.

The objective of this research was to determine the performance characteristics of commercially available edamame cultivars for production in Alabama. Data collected will provide interested growers with a thorough description of each cultivar to include yield, beans per pod, bean weight, plant height, maturity group, days to harvest, and growth habit.

Materials and Methods

Field studies were conducted for two years beginning 27 May 2014, and continuing until 25 Sept. 2015 at the Plant Breeding Unit of the E.V. Smith Research Center (Shorter, AL) (32°29'34.93"N, 85°53'28.45"W). Selected edamame cultivars were grown on a Cahaba fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Hapludult) with a history of successful soybean cultivation, thus no inoculant was applied. Wheat (*Triticum aestivum*) immediately preceded the edamame crop in both years. Separate plots were used in each year of this study and were located approximately 50 m apart. Experimental plots were conventionally managed using pesticides labeled for use in edamame production (Ross, 2013). Soil tests taken prior to each crop indicated that levels of P and K, and the soil pH were within recommended levels, thus no N, P, K or lime was applied. Irrigation was applied at a rate of 2.5 cm weekly through a center pivot irrigation system as necessary to supplement natural rainfall (Table 1). Weather data were collected daily in both years using an onsite weather station (AWIS, 2015).

The experiment was planted in a randomized complete block design (RCBD) consisting of 11 cultivars in 2014, and 10 in 2015, with four replications. Four border rows were seeded with a commodity type soybean (Henderson) around the parameter of the plots. Plots were comprised of two 6 m rows with a 91.4 cm between-row spacing. Plots were tilled to a depth of 15 cm and cultivated prior to seeding to prepare the seedbed. Dual Magnum (S-metolachlor) was applied pre-plant in both crops followed by an application of Select Max ((E)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) midway through each crop. Capture (Bifinthrin) was used as needed to control insect pests. All treatments were seeded at a rate of 148,263 seeds ha^{-1} at a depth of 2.5 cm using an ALMACO four row planter (ALMACO Corp., Nevada, IA). Treatments (and their respective cultivars) were represented by four maturity groups : MG III (Midori Giant, Chiba Green, Butterbean, Sayamusume, and BeSweet 2001), MG IV (Gardensoy 42, Mojo Green), MG V (Mooncake, Lanco, and Gardensoy 51), and MG VI (Owens) These varieties were selected to represent different MG and because some had shown promise in studies outside the southeastern United States (Table 2). Seed were purchased in February 2014 and were stored between the 2014 and 2015 growing season in a seed storage facility at a constant 7° C. Edamame was seeded on 27 May and 2 June in 2014 and 2015, respectively.

Data collection began 14 DAP (Days after Planting). Percentage emergence was determined by counting plants that had reached the V1 stage from two, 3 m row sections within each plot. First bloom data was taken beginning approximately one month after planting and continued until all cultivars had bloomed. Plant height was measured by randomly selecting three plants in each plot at the R6 development stage. Height to first pod was measured concurrently with plant height using the same three plants. Height was measured from soil level to the lowest point of the pod. At R6, two, 3m sections of each plot were cut using a Stihl FS 90 mounted with a Stihl circular saw blade (Stihl Inc. Virginia Beach, VA). Harvest dates varied from mid-August to late September depending on maturity group. Plants were cut at ground level, bagged and removed from the field for hand harvesting.

Pods were graded according to quality standards prior to weighing. This step was done to ensure that only marketable weights were calculated and that tested cultivars met established edamame standards (Konovsky et al., 1994). Only pods that were completely green, with no yellowing contributed to total yield. Any pods that were diseased, damaged, split, or spotted were rejected. To obtain total yield per plot, plants from each plot were stripped of all pods and weighed. To determine beans per pod, pods per plant, and individual bean weight, a representative subsample of five plants was collected from each replicate and stripped of pods. Once pods were removed, they were separated into groups containing one, two, or three beans. Pods in each group were counted and weighed, and then added back to the total harvest to determine yield. Total pods harvested from the five plant subsample were used to determine the average number of pods per plant. Bean weight was determined by hand shelling equal amounts of two and three bean pods to produce 25 beans. The 25 beans, as well as their shells, were weighed separately and recorded.

All data were subjected to analysis of variance procedure (PROC GLM) (SAS Institute, Cary, NC). Data from each crop were analyzed by year due to year interaction ($p<0.001$).

Results and Discussion

Yield

In 2014, total yield of green pods ranged from 10,226 to 15,966 kg ha^{-1} (Fig. 1). The highest yielding cultivars were Gardensoy 51, Midori Giant, and Mooncake, all of which produced yields greater than 15,000 kg ha^{-1}. BeSweet 2001 produced yields significantly lower than 8 of the 11 cultivars evaluated. No statistical differences in yield were found between the eight highest yielding cultivars.

In 2015, yields were slightly lower than in 2014 (Fig. 1). Yields varied greatly among cultivars. The highest yielding cultivars were Mojo Green, Gardensoy 51, and Mooncake at 13,576, 13,195, and 13,092 kg ha $^{-1}$ respectively. The lowest yielding cultivar was the same as in 2014, BeSweet 2001, producing only 2,592 kg ha $^{-1}$. Owens, Midori Giant, Sayamusume, Chiba

Green, Butterbean, and Lanco produced statistically equivalent yields, with weights of 9,122 to 10,378 kg ha $^{-1}$.

The overall reduction in yield between years could most likely be attributed to environmental differences, more specifically air temperature during early reproductive growth. Temperatures at planting, both soil and air, were nearly identical in both years. Total rainfall in both seasons was within 1 cm and was adequate during germination and emergence (Table 1). However, air temperatures were significantly higher during the R1 growth stage in 2015. Average high air temperature was significantly higher ($p > 0.0001$) during the R1 stage was 2.3° C higher in 2015, likely affecting pollen viability and subsequent seed weight (Siebers et al, 2015; Hatfield et al, 2011). Overall temperatures in 2014 were closer to the historic average for the area than in 2015. In 2015, nine of the ten cultivars evaluated had a reduction in yield compared to 2014. Despite the higher temperatures, one cultivar, Mojo Green, produced slightly higher yields (Fig 1). This finding suggests that Mojo Green is more tolerant to high temperatures during early reproductive stages and thus may be better suited for temperatures experienced in the southern U.S.

Yields produced in this study were compared to those obtained in previous work. Sayamusume and Butterbean were previously evaluated in North Dakota and produced a two year average of 7,975 and 9,941 kg ha^{-1} respectively (Duppong and Hatterman-Valenti, 2005). Though lower than the yields in this study (ours were 11,553 and 11,364 kg ha^{-1}), such variation could largely be attributed to environmental conditions. Yields observed in this study compared reasonably well with those found in previous studies (Duppong and Hatterman-Valenti, 2005, Sciarappa et al., 2007, McPherson et al., 2008). However, work by Zhang and Kyei-Boahen (2007) reported yields much higher than those found in this study. In that work the cultivars

Gardensoy 42, Midori Giant, and Moon Cake produced yields more than twice those observed in this study, while Mojo Green produced yields over three times our highest recorded yield. The cause for such large differences in yield cannot be explained.

Generally, yields were lower from edamame in the earliest maturity group (MG III). This result is not unexpected due to the lack of vegetative growth and premature blooming brought on by the short day lengths experienced in the southern United States. Day length at seeding in central Alabama is 14:09 hours, whereas day length in Illinois, where MG III's are recommended is 14:49 hours (Astronomical Applications Department of the U.S. Naval Observatory, 2015). This photoperiodic deficit can have a marked effect on both vegetative growth and yield (Board and Hall, 1984). As a result, the later maturing groups (MG IV, V, and VI) tended to have higher yields than the early maturing group III's.

With the exception of varying percentages of one-seeded pods, all of the cultivars produced edamame yields of marketable quality. Pods were sufficiently long (at least 5 cm) and color was observed to be medium to dark green (Konovsky et al., 1994).

Yield Characteristics

Bean weight. Edamame bean weight was significantly different among cultivars in 2014 (Fig. 2). BeSweet 2001, a low yielding MG III cultivar, produced a 25 bean weight of 30 g. This was more than twice the weight of the smallest beans produced by both Butterbean and Owens (14.5g). Sayamusume, Midori Giant, and Chiba Green produced large beans as well, with 25 bean weights of 27.3g, 26.3g, and 25.8g. The weight of these was not statistically different than that of BeSweet 2001. Gardensoy 51, Lanco, Mooncake, Mojo Green, and Gardensoy 42 produced 25 bean weights which ranged from 20.3 g to 19.0g. These weights were significantly lower than those measured in the two cultivars with the largest bean weights, BeSweet 2001 and Sayamusume.

Bean weights in 2015 were less variable and significantly lower than those found in 2014 (Fig. 2). Mojo Green produced the highest 25-bean weight of 19.1g. This was not significantly different from the 25 bean weights from Sayamusume, Chiba Green, Mooncake, BeSweet 2001, or Lanco. As in 2014, Owens and Butterbean produced the smallest beans, with a weight of 12.1g and 10.6g respectively.

It was hypothesized that the difference in bean weight between years was the result of high air temperatures during the reproductive growth stage in 2015. Average high temperature during the early reproductive stage (R1) in 2014 was 29.7° C (AWIS, 2015). In 2015, the average temperature during the same growth stage was 32.7° C (AWIS, 2015). It has been shown that temperatures in excess of 30° C, particularly during the reproductive stages of development, have a deleterious effect on both pollen viability as well as seed weight (Hatfield et al, 2011). Even short term exposure to high temperatures (3 days and 6° C above ambient) resulted in a 10% yield reduction, a result of reduced pollination and seed weight (Siebers, 2015).

In both years Gardensoy 51, Mooncake, and Lanco, all maturity group V's, produced 25-bean weights that tended to fall in the middle of all cultivars evaluated (Fig. 2). The only MG VI in the study, Owens, produced small beans in both years, ranking near the bottom of all cultivars. One theory as to the difference in bean weight is related to how and where each cultivar was developed. Differences are likely due to the fact that the parental lines of the group III cultivars were selected over hundreds of years for their large seed in the agricultural regions of China and Japan where group III cultivars would be best adapted, whereas the group V and IV are more closely related to commodity type beans developed more recently in the United States that are more suited to southern regions of the country. (Shurtleff and Aoyagi, 2009).

Bean weight in this study was similar to those found in previous work. Sayamusume and Butterbean were evaluated for bean weight previously. Beans produced by Sayamusume and Butterbean were found in one study to be larger than in this study and smaller in a second study (Miles and Sonde, 2002, Duppong and Hatterman-Valenti, 2005). Beans produced by Midori Giant and BeSweet 2001 were larger in this study than in work performed in Virginia in 2008 and 2009 (Carson et al., 2011).

Though bean weight varied throughout the study, all cultivars produced beans of acceptable weight for use as edamame (Konovsky et al., 1994). In order to meet the size standard for edamame, 100 seed must weigh in excess of 30 g (Konovsky et al., 1994). The smallest seeded cultivar in this study (Butterbean), produced a 100 bean weight was 42.4 g, well in excess of the minimum standard.

Pods per plant. Pods per plant appeared to be largely a characteristic of the maturity group of each cultivar (Table 3). Later maturing cultivars had significantly more pods than early maturing cultivars. This was likely due to less vegetative growth by the MG III’s prior to entering the reproductive stage of growth. In addition to the reduction in blooms when subjected to sub-optimal day lengths, the overall reduction in vegetative growth reduces the photosynthetic area required to nourish the developing seed, thus reducing seed weight and quality (Board and Hall, 1984). Premature blooming as a result of sub-optimal photoperiods has been shown to not only reduce overall soybean yield but to reduce seed weight and number of seed per pod (Board and Hall, 1984, Kantolic and Slafer, 2001).

This trend was evident in both years, with beans from the taller MG IV-VI groups producing the most pods per plant. The one notable exception to this was Butterbean (MG III), which produced a high number of pods per plant (99 in 2014 and 64 in 2015). In 2014 and 2015, Owens had the most pods per plant at 124 and 81, respectively. In 2014, Owens had significantly more pods than any other cultivar, while in 2015 pods per plant from Owens was not significantly different than seven other cultivars. In 2014, Sayamusume and Chiba Green, both MG III's , produced less than 40 pods per plant, less than half that of pods produced in many of the MG V and VI groups.

Beans per pod. Cultivar had a great influence on the percentage of three-, two-, and one-bean pods produced. Some cultivars produced greater than 50% three bean pods, whereas others were less than 10% (Table 3). Mojo Green and Butterbean had the highest percentage of three bean pods in both years tested. In 2014, Mojo Green and Butterbean yielded 53% and 50% three-bean pods, significantly higher than any other cultivar. In 2015, Butterbean had the highest percentage of three-bean pods at 51%, followed by Mojo Green at 36%. In both years BeSweet 2001 and Lanco had the lowest percentage of three-bean pods, never exceeding 9%.

As expected, cultivars with a high percentage of three-bean pods tended to produce a lower percentage of both two- and one- bean pods. For most cultivars, two-bean pods were most abundant. Lanco produced the highest percentage of two-bean pods in 2014 with 72%, followed by Gardensoy 42 at 68% and Owens at 66% (Table 3). In 2015, Gardensoy 51 and Lanco yielded the most two-bean pods with 70% and 62% respectively. In both years, the remaining cultivars ranged from 40% to 60% two-bean pods. Cultivars producing the least two-bean pods were Mojo Green and Butterbean, the cultivars that produced the highest percentage of three-bean pods. This was observed in both years of the study.

Because Grade A edamame requires that greater than 90% of pods contain two or three beans, the percentage of one-bean pods should necessarily be below 10% (Konovsky et al., 1994). In this study the percentage ranged from a high of 35% down to as low as 9% (Table 3). In both years BeSweet 2001 had the highest percentage of one-bean pods with 27% and 35% in 2014 and 2015. Midori Giant and Butterbean yielded the lowest percentage of one-bean pods in both years of the study, with an average of 11% and 9% respectively.

Cultivar appears to strongly influence the bean per pod distribution, while MG has little effect. The cultivars, Butterbean and BeSweet 2001, which produced the highest and lowest percentage of three bean pods were both in maturity group III. This finding was consistent with previous work with Butterbean and Sayamusume (Carson et al., 2011). The two cultivars yielded similar percentages of three-, two-, and one-bean pods in different years and different locations, further supporting the importance of genotype on bean per pod distribution.

Plant Characteristics

Emergence Percentage. Seedling emergence was high in 2014, with few significant differences among cultivars (Table 4). Chiba Green had the highest percent emergence (95%). Poorest emergence was found in Owens (61%), which was higher than emergence percentage found in previous studies (Williams et al., 2012). Average percent emergence among all cultivars was 81%. Emergence was lower in 2015 across most cultivars. The reason for this decrease is unclear, but it is unlikely that temperature or rainfall was a contributing factor as they were similar between years. Seed quality was not likely a contributing factor as they were harvested and stored under optimal conditions to guarantee high germination percentages. One factor that may have influenced percent emergence was pest pressure. During both years, minor damage was observed by black cutworms (*Agrotis ipsilon*) which could have easily accounted for the reduced emergence. Lowest emergence in 2015 was measured in BeSweet 2001 (24%), while Mojo Green had highest emergence percentage (82%). Overall emergence average in 2015 was 68.3%.

Plant and pod height. Height at R6 was very consistent within cultivars in both 2014 and 2015 (Table 4). In both years Mooncake was the tallest, averaging 161 cm, followed by Owens at 103 cm. The later maturing group V's and VI's were the tallest, whereas the MG III's were the shortest. With the exception of Mooncake and Butterbean, both of which exhibit an indeterminate growth habit, the height of each cultivar corresponded to the maturity group classification. In both years the shortest cultivar was BeSweet 2001, though only significantly so in 2015.

Height to lowest pod (measured from ground to lowest node on the plant with a pod) was correlated with plant height. The correlation coefficient for 2014 was 0.70 ($p < 0.0001$) and 0.30 ($p < 0.0011$) in 2015. In 2014, Mooncake and Owens had the highest first pod of 16 cm and 12 cm, respectively (Table 4). In 2015, Midori Giant had the highest first pod height (13cm), followed by Owens and Mooncake. This finding was somewhat unusual as Midori Giant was not one of the taller cultivars. BeSweet 2001, the shortest cultivar, had the lowest first pod height of 5 cm in 2014 and 2.8 cm in 2015.

If the crop is to be mechanically harvested, plant size and lowest pod height will become an important consideration to maintain harvest efficiency. If harvesting is done by hand plant size and lowest pod height is not a factor. Plant height and height of lowest pod are important considerations for selecting a cultivar for commercial production. It has been shown that tall plants can have a negative effect on harvest efficiency (Zandonadi et al., 2010).

Although outside the scope of this research, edamame cultivar development through traditional plant breeding has the potential to greatly improve currently available cultivars. The great variability within the tested cultivars may provide opportunities to select desired traits. Though none of the cultivars tested appeared to be ideal, a select combination of the available traits has the potential to produce a superior cultivar. By selecting cultivars that exhibit a short, determinate growth habit, large seeds, a high percentage of three bean pods, and high yield potential, breeders could potentially develop cultivars that are more suitable to the southeastern U.S.

Conclusions

Cultivar selection is of great importance for growers considering edamame as an alternative or new crop. The potential to produce a successful edamame crop depends on a number of factors evaluated in this study. Growers should consider yield and yield characteristics, planned harvest method, as well as consumer preference when selecting cultivars. Often, to spread out harvest dates and risk from environmental factors during key phases of reproductive development, more than one cultivar should be grown. Based on the results of this two year study, the cultivars Chiba Green, Midori Giant, Mojo Green, and Sayamusume possessed the most desirable combination of yield, plant height, individual bean weight, and the percentage of pods containing three beans. Each of these cultivars produced high yields, averaging 12,454 kg ha^{-1} over the two-year study. Although yield is an important factor, bean size and beans per pod can be of equal importance. The four recommended cultivars all produced large beans, with an average individual weight of 0.81g. In addition, the percentage of three-bean pods was high among the four cultivars with an average of 32 %, while yielding a low percentage of the less desirable one-bean pods (15%). Furthermore, the recommended cultivars are maturity group III's and IV's. For that reason they will produce small plants which are well suited for mechanical harvest. The results of this study will help a potential grower to determine the best cultivar for their specific operation and to increase the chances of successfully producing edamame in Alabama. Our results can also be applied to a soybean improvement program aimed at development of edamame cultivars for production in the southeast with improved maturity characteristics and better adaptation.

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II. Isoflavone Content of Edamame [Glycine max (L.) Merrill] as Affected by Cultivar

Abstract

Cultivar selection and environmental factors during the reproductive stages of development have shown significant influence on isoflavone production in soybean/edamame [Glycine max (L.) Merrill] seed. Isoflavone concentrations were determined for edamame cultivars [Glycine max (L.) Merrill] grown in central Alabama during the 2014 and 2015 growing seasons using ultra-high performance liquid chromatography (UHPLC). Isoflavone content was determined by evaluating twelve isomers, three aglycons (daidzein, genistein, and glycitein) and nine glycosides (daidzin, genistin, glycitin; 6”-*O*-acetyldaidzin,-genistin,-glycitin; 6”-O- malonyldaidzin,-genistin,-glycitin). Higher temperatures were recorded in 2015 than in 2014 during the critical R5 development stage. As a result, large differences in total isoflavone content were observed between growing seasons. Overall isoflavone content in 2015 was reduced by 72% from the previous growing season. Cultivars that experienced average high temperatures < 34° C in the R5 stage of development had percent isoflavone reductions as high as 312%. In 2015, high temperatures in the 14 days preceding harvest were highly correlated with isoflavone concentration (P< .0001). This was consistent among edamame cultivars from maturity groups (MG) III, IV, V, and VI. It was found that later maturing cultivars (MG V-VI) benefited from the cooler temperature experienced during September and thus produced higher levels of isoflavones. Based on this study, growers interested in producing edamame with the highest possible isoflavone content must avoid high temperatures during the R5 stage of development.

Introduction

Edamame [Glycine max (L.) Merrill] are vegetable soybean harvested at the R6 (pods 80% full) stage of maturity. Interest in edamame as a healthy snack food has increased significantly in the United States due to its high protein content and purported health benefits (Shurtleff and Aoyagi, 2009). One of the most studied healthful aspects of soy is related to the isoflavone content. Isoflavones are a class of phytochemicals collectively referred to as phytoestrogens, due to structural similarities to estrogen and their mildly estrogenic effects within the body (Franke et al., 1994). Isoflavones have been shown to reduce incidence of hormone-related cancers, such as breast and prostate, as well as gastrointestinal cancers (Messina and Barns, 1991, Wang and Murphy, 1994; Barns et al., 1994, Franke et al., 1994). Many consumers add edamame to their diet as a way to increase isoflavone consumption. Although no specific daily recommendation for the consumption of isoflavones is available, they are widely regarded as being a beneficial dietary addition.

Though edamame is one of the richest sources of isoflavones found in the human diet, it has been shown that not all edamame cultivars are equal in isoflavone content. Total isoflavone content differed by more 138% among cultivars grown in similar conditions in a study conducted in Alabama (Simonne, et al., 2000). Hutcheson, a late maturing commodity type soybean produced seed with an average isoflavone content of 2,280 µg/g, while the advanced experimental line NTCPR93-286 only produced 414 µg/g. This study showed the high degree of variability present among commodity soybean cultivars, however did not evaluate any commercially available edamame. In addition, the study was conducted over a single season, likely negating the effects of seasonal variations often present in field research. Although it is difficult to compare commodity type soybean with cultivars more suited for edamame, the results of this study indicate that cultivar was a significant factor in isoflavone production.

Work by (Carson, 2010) examined isoflavone content among edamame cultivars in Virginia. Unlike the previous study, Carson used commercially available edamame cultivars as opposed to commodity soybean genotypes. While the total isoflavone contents reported in this study appeared to be lower than those found in work by Simonne, et al, 2000, difference among cultivars were clearly evident (Carson, 2010).

In addition to cultivar differences, environmental stresses such as high temperatures during seed development can greatly reduce the concentration of isoflavones produced in the seed. Research has shown that high temperature stress [33/25C (day/night temperatures)] can reduce total isoflavone content by up to 86% (Chennupati et al., 2012). A second study found that increasing temperature from 18C to 23C during seed development resulted in a reduction of isoflavones by 65%. An additional increase of 5C decreased total isoflavone content by about 90% (Caldwell, et al., 2005). Both studies found substantial reductions in isoflavone content as a result of high temperatures, especially when such temperatures were encountered during the R5 (beginning seed formation) stage of development (Chennupati et al., 2012, Caldwell, et al., 2005). For edamame, this reduction is especially important due to the perceived health benefits associated with edamame consumption (Messina and Barns, 1991, Wang and Murphy, 1994, Barns et al., 1994, Franke et al., 1994). Edamame are harvested at the R6 stage of development, prior to peak isoflavone levels seen in the R7-8 (leaves yellow and shed, pods achieve a mature yellow color) stages (Carson, 2010). For this reason, it is important that producers of edamame attempt to address all management options that could potentially affect isoflavone production.

In the south eastern United States, summer temperatures frequently reach the damage threshold mentioned above, and in many instances can exceed them. High temperatures approaching 38C are not uncommon (AWIS, 2016). Historically, July and August are the hottest months, with average day time high temperatures of 32.8C and night time temperatures of 22C (AWIS, 2016). This poses a problem when attempting to produce isoflavone rich edamame. In central Alabama, most soybean are planted in early part of May. Many of the cultivars grown for edamame production are early maturing (MG III), and thus are in the R5 growth stage during the hottest weeks of the year. By establishing which cultivars produce high levels of isoflavones and avoiding exposure to high temperatures during the R5 stage of development, significant increases in isoflavone content could be achieved.

In this study we examined the effects of cultivar selection and production environment on isoflavone content and distribution in seed collected from 11 commercially available edamame cultivars grown in Alabama. Thus, the objective of this research was to determine the isoflavone levels of edamame cultivars that are suitable for production in Alabama.

Materials and Methods

Edamame production

The experiment was conducted for two years beginning 27 May 2014, and continuing until 25 Sept. 2015 at the Plant Breeding Unit of the E.V. Smith Research Center (Shorter, AL) (32°29'34.93"N, 85°53'28.45"W). Selected edamame varieties were grown on a Cahaba sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Hapludult) with a history of successful soybean cultivation, thus no inoculant was applied. Experimental plots were conventionally managed using pesticides labeled for use in edamame production (Ross, 2013). Soil tests taken prior to each crop indicated that levels of phosphorus and potassium, and soil pH were within recommended levels, thus no nitrogen, phosphorus, potassium or lime was applied. Irrigation was applied at a rate of 2.5 cm weekly through center pivot irrigation system as necessary to supplement natural rainfall. Weather data were collected daily in both years using an onsite weather station (AWIS, 2016).

The experimental design was a randomized complete block design (RCBD) consisting of 11 cultivars in 2014, and 10 in 2015, with four replications each. Plots were comprised of two 6 m rows with 91.4 cm between-row spacing. Plots were tilled to a depth of 15 cm and cultivated prior to seeding to prepare the seedbed. Dual Magnum (S-metolachlor) was applied pre-plant in both crops followed by an application of Select Max ((E)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) midway through each crop. Capture (Bifinthrin) was used as needed to control insect pests. All varieties were seeded at a rate of 148,263 seeds ha^{-1} at a depth of 2.5 cm using an ALMACO four row planter (ALMACO Corp., Nevada, Iowa). Four maturity groups were selected for this study: MG III (Midori Giant, Chiba Green, Butterbean, Sayamusume, and BeSweet 2001), MG IV (Mojo Green), MG V (Mooncake, Lanco, and Gardensoy 51), and MG VI (Owens). Seed were purchased in February 2014 and were stored between the 2014 and 2015 growing season in a seed storage facility at a constant 7C. Edamame was seeded on 27 May and 2 June in 2014 and 2015, respectively.

At the R6 stage of development, two, 3m sections of each plot were cut using a Stihl FS 90 mounted with a Stihl circular saw blade (Stihl Inc. Virginia Beach, VA). Harvest dates varied from mid-August to late September depending on maturity groups. Plants were cut at ground level, bagged and removed from the field for hand harvesting. A representative subsample of five plants was collected from each replicate and stripped of pods. A 25 seed subsample was collected and stored at -80° C until processed for isoflavone extraction.

Detailed temperature information was collected via an on-site weather station and recorded daily in the Alabama Mesonet Weather Data site (AWIS, 2016). Data including daily high temperature, low temperature, and daily average temperatures were used to calculate average temperatures during the R5 stage of development. In this study, the 14 days prior to harvesting at R6 was used to determine temperatures experienced during the critical seed filling stage of development.

Isoflavone analysis and Extraction

Total isoflavone content and composition was determined for each of the edamame samples collected during the two year study. Eleven cultivars and four replicate were used in this study for a total of 44 plots. Each cultivar was represented by four individual samples. All edamame samples were collected at the R6 stage of development and stored at -80C until processed for extraction.

Extracting Isoflavones

Edamame samples were placed frozen in a laboratory freeze drier until a constant weight was reached (Lab Conoco, Free Zone 6 plus). Dried samples were removed and finely ground using an electric coffee bean grinder (Hamilton Beach, Fresh Grind). Sample processing was done under yellow light (fluorescent 40w GE). Samples were weighed (0.5g) and placed into a 15ml glass tube along with 5ml of 80% aqueous methanol (Zhang and Schwartz, 2005). Immediately after adding the aqueous methanol, each tube was vortexed for 30 seconds to insure a thorough mix. Tubes were then placed in racks and sonicated (Branson 5510) for 10 minutes at room temperature. After sonicating, samples were placed in a table top shaker and allowed to shake for 2 hours. At the end of 2 hours, samples were centrifuged at 5000 x g for 5 minutes. Next, 2ml of supernatant was collected and filtered using a 0.45µm syringe filter directly into amber colored vials.

Isoflavone Standards

Isoflavone standards were purchased from commercial suppliers of analytical grade compounds. The 6”-*O*-acetyldaidzin,-genistin, -glycitin; 6”-O- malonyldaidzin,-genistin,-glycitin were purchased from Nacalai USA (San Diego, CA). Daidzein and genistein were purchased from ACROS Organics (Springfield Township, NJ). Glycitein was purchased from TSZ Chemicals (West Chester, PA), genistin from BioVisions (Milpitas, California), and Daidzin from ENZO Chemicals (Farmingdale, NY). All standards were supplied in a dry powder form and were of a high degree of purity (< 98%). Standards were dissolved in 80% aqueous methanol. All standards were Vortexed for 1 min to dissolve the standards. It was necessary to sonicate some standards to ensure complete dissolution into aqueous methanol. Standards were stored at 0° C until ready to use.

Ultra-High-Pressure Liquid Chromatography Analysis

Isoflavones were analyzed using a Waters Acquity UHPLC system equipped with PDA detector (210-280 nm). An Acquity UHPLC BEH C18 column (2.1 X 50 mm, 1.7 um particle size) was used for separation. A binary gradient elution consisting of acetic acid (1%) (solution A) and 100% acetonitrile (solution B) were carried out at the flow rate of 0.2 ml/min as follows: 90% A at the first 5 min, then decreased linearly to 82% in 5 min, followed by a decrease linearly to 77% in 14 min. After that, solution A was adjusted to 65% and was held for 5 min, then solution A was adjusted to 90% and held for 6 min for the column equilibration. The gradient elution method produced clear separation of peaks for all twelve isomers (Fig 3).

Statistical Procedures

All data were subjected to analysis of variance procedure (PROC GLM) and correlation procedures (PROC CORR) (SAS Institute, Cary, NC). Means were analyzed using the LSD method at $\alpha = 0.05$. Data from each crop were analyzed separately due to a year by year interaction.

Results and Discussion

Cultivar evaluation

Cultivar was a significant factor in overall total isoflavone content in both 2014 and 2015 ($p = 0.0064$ and $p < 0.0001$), respectively. Isoflavone content ranged from 51.7µg/g in Sayamusume to 509.2µg/g in Mooncake (Table 5). In 2014, Mooncake had the highest concentrations of total isoflavones, statistically equal to that measured in Gardensoy 51, the cultivar producing the highest isoflavone content in 2015. In 2015, Owens also had seed with high isoflavone content, equal to that measured in Gardensoy 51. Other cultivars tended to produce lower isoflavone concentrations in both years of the study. In both years, the cultivar Sayamusume produced seed with isoflavone content that ranked near the bottom of all cultivars tested, in both years.

While significant variation existed in isoflavone content among cultivars, it was important to compare cultivars that were harvested on the same date to account for environmental conditions during the R5 stage of development (14 days prior to harvest). A total of four harvests were made to account for the different maturity groups represented in the study. This was done to show that differences in isoflavone content were in fact a function of genotype, and were not simply the result of high temperatures. When an analysis was made among cultivars within the same harvest group statistical differences remained. For example, in the first group of cultivars harvested in 2014, Midori Giant had isoflavone content significantly higher than other cultivars in the same group, significantly higher than found in Mojo Green, BeSweet 2001, Sayamusume, and Chiba Green. In the third group harvested, Gardensoy 51 had significantly higher isoflavone content (418.92 μg/g) than that measured in Gardensoy 42 (105.2 μg/g).

In 2015, comparisons within harvest groups revealed statistical differences similar to results observed in 2014. Among the first group of cultivars harvested in 2015, Midori Giant again had significantly higher isoflavone content than observed in Butterbean, BeSweet 2001, and Sayamusume. Differences were found among cultivars in the fourth harvest as well. Gardensoy 51, the cultivar with the highest isoflavone content in 2015 was found to be significantly higher than Lanco, the third highest of 2015.

In both years, 6”-O- malonyldaidzin and 6”-O- malonylgenistin were the isoflavone conjugants found in the highest concentration in most of the cultivars evaluated. This finding was consistent with results reported in work by Simonne et al. (2000) and Carson (2010). Though glycoside derivatives are not biologically available in their current form, hydrolysis in the digestive tract or through processing of the edamame converts these glycosides into the more biologically active aglycons (Yonemoto-Yano et al., 2014; Fujita et al., 2015; Yerramsetty et al., 2014). In the case of Mooncake, the cultivar with the highest isoflavone content in 2014, over 47% of the total isoflavone content originated from 6”-O- malonyldaidzin and 6”-O- malonylgenistin each contributing 20% and 27% respectively (Table 5). The cultivar with the highest isoflavone content in 2015, Gardensoy 51, had a higher percentage of malonyl isoflavones with 6”-O- malonyldaidzin and 6”-O- malonylgenistin accounting for 80% of the total isoflavone content. 6”-O- malonyldaidzin contributed 39% while 6”-O- malonylgenistin contributed 41% (Table 1).

The aglycone isoflavones were found in very low quantities which were consistent with finding reported by Simonne et al. (2000) and Carson (2010). In many cases the content was below detection limits. Of all cultivars evaluated, none had detectable levels of 6”-*O*-acetylglycitin or 6”-*O*-acetylgeneistin. All cultivars had low, but detectable levels of 6”-*O*-acetyldaidzin; however they contributed little to the total isoflavone content (< 2%).

Effect of Temperature on Isoflavone Content Discussion

As discussed previously, temperature during the R5 stage of development can have a tremendous impact of total isoflavone content. In this study, the effect of temperature during the R5 stage of development was quantified and compared between years. Temperature data, including daily high, low, and average temperature were compared to total isoflavone content for each cultivar during the two year study. Between the 2014 and 2015 seasons, significant reductions in total isoflavones were documented (Table 5). While overall growing conditions during each season were essentially the same, the higher average temperature experienced in 2015 during seed development had a significant effect on isoflavone content (Table 6). From the data collected comparing temperature to isoflavone content, all cultivars exposed to temperatures in excess of 34°C were severely affected (Fig 4 and 5). The cultivars Butterbean and BeSweet 2001 had a greater than 74% reduction in isoflavone content between 2014 and 2015.

Data collected during the 2014 season seemed to indicate that temperature was not a large factor in total isoflavone content in the 11 cultivars evaluated (Table 5); (Fig 5). Correlations between daily high temperature, daily low temperature, and average temperatures were weak and of little significance (Table 7). However, data collected in 2015 demonstrated a highly correlated and significant correlation between daily high temperatures, daily low temperatures, and average temperatures (Table 7) (Fig). This finding is likely the result of significantly higher temperatures during the R5 stage of development during the 2015 growing season. It appears that a critical high temperature threshold exists at which point isoflavone production within the seed plummets. It was further hypothesized that the weak or non-existent temperature correlation in 2014 was the result of the critical high temperature not being reached during the growing season. While further research would be needed to determine more precisely the temperature at which isoflavones are significantly inhibited, it appears in this study temperature greater than 32.9° C resulted in reductions in total isoflavones.

In summary, this study has shown that edamame cultivar selection and temperature during R5 can play an important role when attempting to produce edamame rich in isoflavones. It was clear from the two year study that significant variation exists as a function of genotype. The information collected in this study, regarding the genotypic influence on isoflavone content could be used as a screening tool in future edamame breeding programs. In some cases, cultivar selection alone can account for differences up to 380% when produced under identical conditions. This level of contrast was found between Midori Giant and Sayamusume in the 2015 season. In addition to the contribution genotype has on isoflavone content, the photoperiodic nature of edamame can also be used to avoid periods of extreme heat. Temperature correlations in this study determined that cultivars which matured later in the season (MG V-VI), avoided the extreme temperatures commonly experienced during August and were therefore more likely to produce seed with higher levels of isoflavone. While production schedules and market demand play a large role in planting dates and cultivar selection, producers interested in marketing a product rich in isoflavones should carefully consider cultivar selection, maturity date, and environmental conditions when planning their up coming season.

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III. Evaluation of Sensory Attributes of Selected Edamame Cultivars

Abstract

A study evaluating consumer preference of six locally grown edamame cultivars was conducted during the summer of 2015 and 2016. Four taste evaluations were conducted within a 20 mile radius of Auburn University with a total of 250 subjects. Participants sampled and ranked various sensory attributes including taste, sweetness, external color and overall likeness. Preferences among cultivars were present due to both textural and visual rankings. Sayamusume, Midori Giant, Mojo Green, and Chiba Green were preferred over both Gardensoy 51 and Owens in overall likeness. Owens and Gardensoy 51 ranked statistically lowest in taste and texture. Owens ranked lowest in external appearance due to the presence of dark pubescence on their pods.

Introduction

Edamame [*Glycine max* (L.) Merr.] is vegetable soybean harvested and consumed at the R6 development stage. The growing popularity of edamame as a healthy snack food has led to increased interest in edamame production from soybean producers across the southeast. In recent years, there has been an increased demand and broader acceptance of edamame. Edamame is currently the second most consumed soy food, behind soy milk, in the United States (Soyfoods, 2014). In 2013, it was estimated that the United States consumed between 22,700-27,270 tonnes of edamame (Nuss, 2013). This increased demand is expected to continue as consumers look for healthier, lower cost sources of protein to add to their diet.

While edamame is an excellent source of plant based protein, it is also a complete protein, containing all the essential amino acids in the human diet (Velasquez and Bhathena, 2007). Edamame is one of only a handful of plant-based foods that contains all the essential amino acids. In addition, edamame is high in isoflavones (Wang and Murphy, 1994). Isoflavones have been reported to reduce the incidence of certain cancers, and are also powerful antioxidants (Simonne et al., 2000, Franke et al., 1994, Wang and Murphy, 1994).

Potential health benefits have long been established for edamame, however little work has been done to investigate the organoleptic characteristics of different cultivars. Taste and related sensory attributes of a food product are often a decisive factor in whether or not to consume or purchase a product. This is especially true when introducing an unfamiliar product such as edamame. In order to assess the specific preferences that exist within a local population it is necessary to have individuals evaluate and rank various sensory attributes. In previous studies consumer liking and sensory evaluations have been conducted using a nine point hedonic scale. Sensory components evaluated varied between studies based on the specific objectives of each. Both studies did however focus on external appearance, firmness, taste, sweetness, overall likeness, and willingness to purchase (Kelley and Sanchez, 2005). Attribute such as “beaniness” and “nuttiness” were also attempted but could not be differentiated among cultivars (Wszelaki et al, 2005). In both studies the overall time to complete each tasting was between 10-15 min. In a fast paced environment, time limits will likely be an important factor.

In this study we obtained data from 250 participants in order to evaluate sensory preferences of six edamame cultivars that had previously shown the potential for commercial production in the south eastern United States (Ogles et al., 2016). Thus, the objectives of this study were to 1) Determine consumer taste preference among selected edamame cultivars, 2)

Collect demographic information in order to determine preferences among different groups within our geographical area.

Materials and Methods

A two year sensory evaluation study was initiated in July 2015 and ended in August 2016. The study consisted of a total of four taste testing events: two at the Market at Heritage Ag Park on the Auburn University campus, one at the Market on Broadway in Columbus Georgia, and once at Funchess Hall on the Auburn University campus. Edamame used in this study was grown at the Plant Breeding Unit of the E.V. Smith Research Center (Shorter, AL) (32°29'34.93"N, 85°53'28.45"W). Experimental plots were conventionally managed using pesticides labeled for use in edamame production (Ross, 2013). Irrigation was applied at a rate of 2.5 cm weekly through a center pivot irrigation system as necessary to supplement natural rainfall. Cultivars used in this study were Sayamusume (Territorial Seed Co.), Midori Giant (Wannamaker Seeds), Chiba Green (Wannamaker Seeds), Owens (Cyrille Young (Farmer Direct), Mojo Green (Sow True Seed), and Gardensoy 51 (University of Illinois). These cultivars were selected due to successful harvests in a prior study evaluating agronomic traits of edamame cultivars in the same location (Ogles et. al., 2016).

Once harvested, the unshelled edamame was washed to remove any field debris and blanched for two minutes in boiling water before being drained and immediately immersed into iced water. Once the edamame had cooled, it was drained, placed in labeled zip top bags, and placed in a deep freezer (-18°C) until ready to use. Edamame remained frozen until being transported to the taste evaluation on ice. Once on site the frozen edamame was placed in unsalted boiling water for an additional two to three minutes to defrost and to finish cooking.

When finished, the prepared edamame was placed in an individually labeled electric warming container. Each edamame cultivar was kept at a constant temperature for the duration of the testing.

As participants arrived they were presented with a disposable lunch tray with six individual compartments. Participants had no prior knowledge of cultivar appearance or order within the tray. Each compartment was numbered 1-6 and contained 2-3 representative edamame pods of each of the six cultivars (Fig 6). Participants were instructed to begin with compartment 1 and to go in numerical order when completing the test. Due to the possibility of an order effect on sensory perceptions or possible tasting fatigue, the trays were randomized in a counterbalanced fashion. This was done by developing a Latin square design that insured that each of the six cultivars appeared once in each of the six possible compartments in each cycle (Fig 6). The cultivar order for each tray was determined by a letter on the bottom of each tray that corresponded to the correct sequence. This additional randomization was initially not disclosed to participants; however more observant participants noticed external differences between neighboring trays. For this reason participants were instructed not to compare samples with neighboring testers.

Participants were given a sensory acceptance survey at the beginning of the test. Subjects were instructed to rate seven sensory characteristics of each of the six edamame cultivars on a nine point hedonic scale. The characteristics included external color, bean color, taste, sweetness, texture, peelability, and overall likeness. At the end of the sensory acceptance survey, subjects were asked to complete a short demographic questionnaire. During the taste test, participants were give unsalted crackers and bottled water to cleanse their palette between samples. Salt and other seasonings were avoided throughout the study to ensure only the characteristics and taste of the edamame cultivars was rated without distraction.

Sensory data was collected from 250 participants during the two year study. As with many taste evaluation surveys, the data collected was found to be not normally distributed. For this reason a nonparametric statistical analysis technique was used. Ranked data was generated to reduce variation inherently present in participant scoring. A program was written to change each consumer rating into rankings, with 1 being best and 6 being the lowest. For example, if a consumer had one cultivar as a 9, and the others as less than 9, the variety with a 9 gets ranked 1 and the rest are ordered down to 6 (lowest rank). If two varieties score the same high mark, they each get 1.5 and so on (skipping spaces for the next one to get to 3 and so on) Rankings helps minimize the problem of people who are more or less likely to go to high or low ends of the scales. The ranked data was used to determine significance at an alpha level of 0.05 (Robbins, 2003). Data from both years were combined and analyzed as one data set due to no significant difference between years.

In two of the taste evaluations nominal gifts and soft drinks were offered to subjects for their participation. Subjects had a choice between a soybean shaped stress balls or a can cooler. Both gifts were printed with an edamame logo to promote and advertise locally grown edamame. The monetary value of each gift was less than $2.00.

Results and Discussion

Consumer preference can be difficult to ascertain when comparing very similar products, thus the need for large sample populations. In this study, clear preferences were noted among the six edamame cultivars evaluated. When evaluating cultivars for “Overall Likeness” it was found that Sayamusume, Midori Giant, Chiba Green and Mojo Green were the preferred cultivars. Owens and Gardensoy 51 ranked lowest in overall likeness (Fig. 7). Sayamusume and Midori Giant were numerically higher than the two other preferred cultivars; however they were not statistically different. This trend continued throughout the other six sensory attributes on which the edamame was scored. Owens and Gardensoy 51 were ranked lowest in peelability, texture sweetness, taste, bean color, and pod appearance. Few differences were seen among the other four cultivars.

Participants reported that both Owens and Gardensoy 51beans were harder than other cultivars evaluated. Their texture was found to be undesirable by most participants and was additionally described as being both "starchy" and "mealy". Because significant differences were immediately evident in consumer preference, the question was raised as to whether the textural differences may be the result of the preparation method (not cooked enough) or as the result of differences in growing condition between years. Cooking time was consistent among cultivars and was not likely a factor in textural differences. This was further demonstrated when sensory data from all four taste test and throughout both years were statistically equivalent. Texture was not significantly different among the other four cultivars, Sayamusume, Midori Giant, Chiba Green and Mojo Green.

Because most edamame is served in the pod, the ability to peel and eat without difficulty is an important attribute. Participants rated the ease in which each edamame pod could be peeled. The results indicated that Owens and Gardensoy 51 produced the most difficult pods to shell among all cultivars evaluated. It has been hypothesized that the ability to extract the edamame from the pod is related to the size of the bean within. Coincidentally, both Owens and Gardensoy 51 tend to produce smaller pods and beans when compared to the other cultivars and both consistently ranked lowest in peelability. Although the effect of bean size on easy of shelling was not evaluated in this study, future studies may be able to answer this question.

Taste is a difficult attribute to quantify. Taste is highly subjective and subject to inherent bias from each of the participants in the study. With that said, taste preferences were split into two significantly different groups; 1) Owens and Gardensoy 51, and 2) Sayamusume, Midori Giant, Chiba Green and Mojo Green. Participants tended to score Owens and Gardensoy 51 in the “neither like nor dislike” range of the 9 point scale, while the other cultivars were scored in the “slightly like” range. No cultivar was ranked in the “dislike” range of the scale indicating that all cultivars had acceptable taste.

Visual perception is critical when evaluating an unfamiliar food product. This was shown to be a significant factor among consumers when edamame cultivars were compared. In the category of external appearance, Owens was always ranked lowest (Fig. 8). Of the six cultivars evaluated, all had light colored pubescence with the exception of Owens, which had darker brown pubescence. Almost without exception participants disliked the experience of the fuzzy, brown pubescence found on all Owens pods. Participants tended to score Owens in the “dislike slightly” range whereas other cultivars were ranked in the “slightly like” range. Of all the sensory attributes participants were ask to evaluate in this study, none produced as immediate or as strong of a reaction as did the appearance of Owens pods. Opinions were so strong with some participants it is believed to have affected their opinion about unrelated characteristics such bean color and taste. A future study may evaluate the same sensory components with shelled edamame to eliminate the potential for bias as a result of the darker pubescence.

Demographic information was collected from all participants that included gender, age, level of education, income, whether they had tried edamame before, and their likelihood to purchase. After statistical analysis it was found that the gender of participants had little or no effect on sensory perception of the cultivars evaluated. Both males and females preferred Sayamusume and Midori Giant in overall likeness and ranked Owens and Gardensoy 51 lowest. Some variability was observed between males and females and among cultivars when comparing numerical means, however, no statistical differences were noted.

It was hypothesized that participants familiar with edamame may be more discriminating when given six cultivars to compare and contrast. It was found however that prior experience with edamame had no significant influence on which cultivars were preferred. In all cases, those with prior experience with edamame ranked the cultivars the same as individuals with no familiarity with edamame. This finding makes selecting an edamame cultivar for commercial production easier as preferences are likely to be consistent across a wide range of the population.

Age, level of education and income were found to not be significant when rating edamame cultivars. Again, this lack of specificity in opinion is considered a benefit when determining a cultivar to take to market. Ideally a cultivar offered for retail sale would have broad appeal to many customers.

Upon completion of the taste evaluation, participants were asked to rate their likeliness to purchase. Participants were asked to choose one of five levels describing their likelihood to purchase edamame in the future. The levels to select from were very likely, somewhat likely, neutral, somewhat unlikely, and very unlikely. Of the 250 participants in the study, 52% reported being very likely to purchase and 32% reported being somewhat likely to purchase (Fig 9). A total of 11% reported that they were neutral. A total of 4% of participants reported that they were either very unlikely or somewhat unlikely to purchase. An overwhelming 84% of the participants in the study reported being likely to purchase edamame after tasting it.

Edamame is an exciting new specialty crop that is gaining broad acceptance in the United States. In addition to being a healthy snack food, edamame has the potential to become a new cash crop for growers in the southeastern U.S. Currently, 95 percent of edamame consumed in the United States is grown in China and imported (Roseboro, 2012). Based on the amount of edamame that is currently being grown in the United States, it would take an increase of more than five times just meet domestic demand. It is important to note that these figures do not take into account the steady increase in demand that has been seen in the past decade. This increase in demand for edamame provides enterprising growers a huge opportunity to enter a new and emerging market. Data collected in this study can be utilized by growers, processors, and consumers to ensure their cultivar selection will meet expectations. Many factors go into selecting an edamame cultivar for production. Agronomic characteristics such as yield, days till maturity and bean size are now available for many commercial edamame cultivars. However, little or no information on the organoleptic characteristics of these popular cultivars has existed publicly until this study. With this data, growers and processer will be able make selection on agronomic characteristics as well as the less tangible, but equally important sensory attributes possessed by each cultivar.

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IV. Potassium Fertilization Effects on Selected Edamame [*Glycine max* (L.) Merr.] Cultivars in Central Alabama

Abstract

Edamame [*Glycine max* (L.) Merr.] is vegetable soybean harvested and consumed at the R6 development stage. Although commodity type soybean is grown extensively throughout the United States, little research has been done addressing the plant nutrient requirements of cultivars grown for edamame. Current potassium fertility recommendations are made based on research in commodity soybean with little regard to edamame production. Thus, the objective of this study is to evaluate the effect of increased potassium rates on three selected edamame cultivars in central Alabama. Cultivars include Midori Giant and Chiba Green (MG III) and Owens (MG VI). Cultivars were planted in replicated plots at the Field Crops Unit in Tallassee, AL in June 2015 and June 2016. Data was collected on emergence, plant height at maturity, leaf potassium content, weight of 25 shelled beans, pods per plant, beans per pod, and total yield. In 2015, rates of potassium had no significant effect on yield in any of the cultivars evaluated. A slight increase in yield was seen with Midori Giant at the 82 kg K ha^{-1} rate; however the gains in yield disappeared with increasing rates suggesting that potassium rates were not the dominate factor. The 2016 crop followed much the same trend as the previous year's data. The results of this study showed no increase in seed size in either year. Based on this two year study evaluating potassium rates in edamame, it is clear that the current recommendations for commodity soybean accurately mirror the requirements of the three selected edamame cultivars.

Introduction

Edamame [Glycine max (L.) Merr.] has been consumed as a snack food in China and much of East Asia for generations (Shurtleff and Aoyagi, 2009). Traditionally boiled and served in the pod, edamame holds a place in East Asian cuisine much like peanuts and potato chips in Western culture. Often served as an appetizer or as an accompaniment to beer, edamame is a staple of many diets.

In recent years, the mildly sweet taste of edamame has gained wider acceptance in the United States as an alternative snack food, often taking the place of highly processed, less healthy options (Soyfoods, 2014). The health benefits of edamame have been reported for hundreds of years, dating back to the early 1600's (Shurtleff and Aoyagi, 2009). The *Runan pushi* [An account of the vegetable gardens at Runan] by Zhou Wenhua first documented that edamame could "kill bad chi", reduce edema, reduce bad blood, and is an antidote for poisonous drugs (Shurtleff and Aoyagi, 2009). While many of the early medicinal benefits of edamame have since been disproven, modern research suggests that compounds found in edamame (isoflavones) may prevent certain cancers and provide other health benefits (Messina and Barns, 1991, Wang and Murphy, 1994, Barns, et al., 1994, Franke et al., 1994,). Edamame is an excellent source of plant based protein providing all the amino acids necessary in the human diet (Velasquez and Bhathena, 2007). In addition, 1 cup of shelled edamame contains 676 mg of potassium, roughly one and a half the potassium found in a medium banana (S F Gate, 2016).

As a result of the increased demand for edamame, domestic growers are looking to add edamame as a potential specialty crop. Historically, the vast majority of edamame sold in the United States is imported from China, by some estimations greater than 95% all edamame sold (Roseboro, 2012). Commodity type soybeans, those grown for processing and animal feed, are produced extensively throughout the United States with great success. However, very little edamame is produced domestically. For that reason much of what is known about edamame production has been gleaned from research in commodity soybean production. This has been the case in regards to nutrient management. Potassium, the most abundant cation in plants, and the nutrient associated with water relations, enzyme activation, and photosynthesis has been thoroughly researched and documented in commodity soybean, however, a paucity of information exists for edamame (Pettigrew, 2007). In some cases the information gained from commodity soybean production translates well over to edamame, in other cases it does not. Much of the fertility research done in edamame has focused on nutritional value and less on agronomic variables. While important, these studies provide little information regarding yield and yield components.

Many studies have evaluated potassium rates in commodity soybean, attempting to establish optimal soil levels. Recent studies have evaluated yield and growth components using modern commercially available soybean cultivars. Work by Parvej et. al., 2015, showed that soybean grown in a lower potassium soil produced lower yields as a result of fewer pods and smaller seed size. In contrast, soybean grown on soils containing higher levels of potassium produced more pods, larger seed, and higher yield. It is important to note that soil potassium levels in this study were not extremely low or extremely high (61 and 91 kg^{-1} ha respectively) indicating a narrow range in which small increases in soil extractable potassium can produce significantly higher yields. Similar yield increases were reported as a result of improved seed size and more pods per plant by Pettigrew in 2008. In high potassium soils, more pods per plants were noted along with more seed per pod. This is an important finding as edamame is often graded to ensure that at least 90% of the pods contain at least two or three seed per pod

(Konovsky et al., 1994). In addition, seed size is critically important for edamame. In order to meet the size standard for edamame, 100 seed must weigh in excess of 30 g (Konovsky et al., 1994).

While the vast majority of information on edamame fertility is obtained indirectly through commodity soybean research, work by Oliveira et al., 2012, looked specifically at potassium fertility in edamame. The study evaluated a number of factors relating both to growth and nutritional information in response to 0, 50, 100, 150, 200% of recommended rate of potassium. The single cultivar chosen for the study, JLM019, was obtained in Brazil and is virtually unavailable in the United States. In general, no statistical differences were seen in this study,with the exception of seeds per plant. The number of seed per plant was found to be significantly higher at the highest potassium rates, suggesting that an increase of 50 to 100% of the recommended potassium rates may be economically feasible due to the high value of the crop.

Based on research done in both commodity soybeans as well as in edamame, the potential to improve yields through increased rates of potassium may be a real possibility. In addition to increased yields, the potential to produce larger seed along with more seed per plant has been shown in previous research. Thus the objectives of this research is to determine the effects of increased potassium rates on edamame yield, seed size, pods per plant, plant height, emergence, and leaf K. Data collected will provide up-to-date potassium fertility information for growers of this new specialty crop. This study evaluated the agronomic response to increasing rates of potassium in three commercially available edamame cultivars grown in central Alabama.

Materials and Methods

Field studies were conducted for two years beginning 4 June 2015, and continuing until 14 Sept. 2016 at the Field Crops Unit of the E.V. Smith Research Center (Shorter, AL) (32° 25.634'N, 85°53.279'W). Three selected edamame cultivars were grown on a Cahaba fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Hapludult) with a history of successful soybean cultivation, thus no inoculant was applied. Wheat (*Triticum aestivum*) immediately preceded the edamame crop in both years. Separate plots were used in each year of this study and were located approximately 100 m apart. Experimental plots were conventionally managed using pesticides labeled for use in edamame production (Ross, 2013). Soil tests were taken prior to each crop to determine the overall nutrient status of the soil and to insure that K levels were within recommended levels. This was done to ensure that additional K fertility applications would raise K levels in the soil to above current recommendations Soil potassium levels were reported at 95 kg ha^{-1} for both fields used in the study. Based on the current fertility recommendations for commodity soybean, the level of 95 kg $ha^{-1}$was higher than the recommended 90 kg ha^{-1} (Mitchell and Huluka, 2012) and required no supplemental K fertilizer. Reports further indicated that levels of P, Ca, Mg, and the soil pH were within recommended levels, thus no N, P, Ca, Mg or lime was applied. Irrigation was applied at a rate of 2.5 cm weekly through a center pivot irrigation system as necessary to supplement natural rainfall (Table 6). Weather data were collected daily in both years using an onsite weather station (AWIS, 2015).

The experiment was planted in a randomized complete block design (RCBD) consisting of three cultivars, four K fertility rates, with four replications. Potassium fertilizer in the form of KCl was broadcast pre-plant at a rate of 0, 55, 82, and 110 kg K ha^{-1}. Four border rows were seeded with a commodity type soybean (Henderson) around the parameter of the plots. Plots were comprised of two 6 m rows with a 91.4 cm between-row spacing. Plots were tilled to a depth of 15 cm and cultivated prior to seeding to prepare the seedbed. After fertilizer application, plots were cultivated to incorporate the fertilizer into the top four to six inches of soil. Dual Magnum (S-metolachlor) was applied pre-plant in both crops followed by an application of Select Max ((E)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) midway through each crop. Capture (Bifinthrin) was used as needed to control insect pests. All treatments were seeded at a rate of 148,263 seeds ha^{-1} at a depth of 2.5 cm using an ALMACO four row planter (ALMACO Corp., Nevada, IA). The three cultivars represented two different maturity groups MG III (Midori Giant and Chiba Green), and MG VI (Owens). These cultivars were selected to represent different MG and because they showed promise in a study conducted on a nearby research unit (Table 2). Seed were purchased in March 2015 and were stored between the 2015 and 2016 growing season in a seed storage facility at a constant 7 C. Edamame was seeded on 4 June and 25 May in 2015 and 2016, respectively.

Data collection began 14 DAP (Days after Planting). Percentage emergence was determined by counting plants that had reached the V1 stage from two, 3 m row sections within each plot. First bloom data was taken beginning approximately one month after planting and continued until all cultivars had bloomed. Leaf samples were collected at between the R1 and R2 stage of development. Plant height was measured by randomly selecting three plants in each plot at the R6 development stage. Height to first pod was measured concurrently with plant height using the same three plants. Height was measured from soil level to the lowest point of the pod. At R6, two, 3 m sections of each plot were cut using a Stihl FS 90 mounted with a Stihl circular saw blade (Stihl Inc. Virginia Beach, VA). Harvest dates varied from mid-August to late September depending on maturity group. Plants were cut at ground level, bagged and removed from the field for hand harvesting.

Pods were graded according to quality standards prior to weighing. This step was done to ensure that only marketable weights were calculated and that tested cultivars met established edamame standards (Konovsky et al., 1994). Only pods that were completely green, with no yellowing contributed to total yield. Any pods that were diseased, damaged, split, or spotted were rejected. To obtain total yield per plot, plants from each plot were stripped of all pods and weighed. To determine beans per pod, pods per plant, and individual bean weight, a representative subsample of five plants was collected from each replicate and stripped of pods. Once pods were removed, they were separated into groups containing one, two, or three beans. Pods in each group were counted and weighed, and then added back to the total harvest to determine yield. Total pods harvested from the five plant subsample were used to determine the average number of pods per plant. Bean weight was determined by hand shelling equal amounts of two and three bean pods to produce 25 beans. The 25 beans, as well as their shells, were weighed separately and recorded.

All data were subjected to analysis of variance procedure (PROC GLM) (SAS Institute, Cary, NC). Data from each crop were analyzed by year due to year interaction $p<0.001$).

Results and Discussions

Yield

Yield data were analyzed separately due to a significant year x cultivar interaction. In 2015, rates of potassium had no significant effect on yield in any of the cultivars evaluated (Fig 10). A slight increase in yield was seen with Midori Giant at the 82 kg K ha^{-1} rate; however the gains in yield disappeared with increasing rates suggesting that potassium rates were not the dominate factor. The 2016 crop followed much the same trend as the previous year's data. The one notable exception was that Midori Giant showed a significant decrease in yield between the 0 and 55 kg K ha^{-1} rate (Fig 11). This finding is difficult to explain as a function of potassium fertility rates and was likely due to variation in the field or feeding damage from whitetail deer (Odocoileus virginianus). The lack of a yield response to potassium rates above those currently recommended in commodity soybean production suggests that potassium requirements for these edamame cultivars mirror those in commodity soybean production.

Seed Size

Previous research found that higher levels of soil extractable potassium may have a positive influence on seed size in commodity soybean (Parvej et. al., 2015; Pettigrew, 2008). The results for this study showed no increase in seed size in either year (Fig 12 and 13). In both 2015 and 2016, the largest seeds were produced by Chiba Green, followed by Midori Giant and Owens (Fig 12 and 13). This finding is consistent with previous work evaluating genotypic characteristic of commercially available edamame cultivars and is believed to be completely unrelated to potassium rates (Ogles et al., 2016). While no increase was seen in seed size, it is important to note that all cultivars produced seed of acceptable size for edamame (Konovsky et al., 1994).

Plant Height

Plant height was not significantly affected by potassium rates in either year of the study. Chiba Green was significantly shorter than both Midori Giant and Owens; however this was a function of genotype and not related to potassium fertility.

Pods Per Plant

Few differences were observed in the distribution of three, two and one seeded pods as affected by potassium rate. One exception was found in the distribution of three bean pods in the Owens cultivar. It was found that the percentage of three bean pods were significantly lower at the highest potassium rate (110 kg K ha^{-1}). The percentage of three seed pods was 35% at the 0 kg K ha^{-1} and 12% at the 110 kg K ha^{-1} rate. No significance differences were found in the percentage of two bean pods in either year or among cultivars.

Emergence Percentage

Emergence was not significantly affected by potassium rates in either year of the study (Table 8). While no negative effects were observed as the result of phytotoxicity at the highest fertility rates, potassium rates had no positive effects on stand establishment. All three cultivars had acceptable germination rates resulting in plants stands in of nearly 80%.

Leaf Potassium

Although significant differences in leaf potassium content were minimal, leaf potassium content did seem to increase with higher rates of applied potassium. The only significant difference was found in the second year samples from Owens. In this case, plants grown in plots receiving the lowest rate of potassium (0 kg K ha^{-1}) were significantly lower in leaf potassium content than plants grown in plots receiving the highest rate (110 kg K ha^{-1}). Though statistically significant, it appears that potassium leaf content has little effect on plant growth and development so long as recommended levels of potassium are maintained in the soil.

Conclusions

Based on this two year study evaluating potassium rates in edamame, it is clear that the current recommendations for commodity soybean accurately mirror the requirements of the three selected edamame cultivars used in this study. While it is possible that other cultivars could have different potassium requirements, we found little evidence to support this in the cultivars evaluated in this study. The cultivars used in this study showed no sustained increases in yield, seed size, plant height, leaf potassium content or emergence percentage with increasing rates of potassium fertilizer. Based on the results of this study, it would be difficult to justify the cost associated with adding additional potassium above that currently recommended. For these reasons, it is recommended that current potassium recommendations in commodity soybean be applied to cultivars used in edamame production as well.

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V. Appendix

Table 1. Climate data for each growing season (May-Sept) as recorded by the on-site weather station located at E. V. Smith Research Unit in Shorter, Alabama.

	Year	
	2014	2015
Average air temperature (C)	25.0	25.7
Average soil temperature (C, at10 cm)	28.2	27.3
Average air temperature (C, during R1)	(6/24-7/24) 31.0	(7/1-7/31) 33.3
Evapotranspiration (ET mm/day)	0.21	0.23
Rainfall total (cm)	33.6	32.8
Day length at seeding (hours)	14.1	14.3

Table 2. Edamame varieties, seed sources, and maturity groups for each variety evaluated.

Variety	**Source**[†]	**Maturity Group**
Midori Giant	Wannamaker Seeds	III
Chiba Green	Wannamaker Seeds	III
Sayamusume	Territorial Seed Co.	III
Butterbean	Johnny's Selected Seeds	III
Lanco	Southern Exposure Seed Exchange	V
Moon Cake	Southern Exposure Seed Exchange	V
Mojo Green	Sow True Seed	IV
BeSweet 2001	Stokes Seed	III
Owens	Cyrille Young (Farmer Direct)	VI
Gardensoy 51	University of Illinois	V
Gardensoy 42	University of Illinois	IV

[†] Wannamaker Seed – Saluda, NC, Territorial Seed Company – Cottage Grove, OR, Johnny's Selected Seeds - Winslow, ME, Southern Exposure Seed Exchange – Mineral, VA, Sow True Seed - Ashville, NC, Stokes Seeds Inc. – Buffalo, NY.

Table 3. Pods per plant, bean per pod distribution, and estimated maturity group of each variety. Means within a year and column followed by the same letter are not statistically different as determined via means separation at alpha= 0.05.

Variety	Pods plant^{-1}		Three bean pods		Two bean pods		One bean pods		Estimated MG[‡]
	no.		percent						
	2014	2015	2014	2015	2014	2015	2014	2015	
Owens	124 a	81 a	21 c	20 cd	66 ab	57 ab	13 cde	23 ab	E- VI
Butterbean	99 b	64 ab	50 a	51 a	41 d	40 c	9 e	9 b	III
Lanco	88 bc	52 ab	7 d	9 de	72 a	63 ab	22 ab	29 ab	V
Gardensoy 51	77 bcd	62 ab	18 c	18 cde	65 abc	70 a	17 bcde	13 b	V
Midori Giant	47 ef	43 b	29 b	27 bc	60 bc	61 ab	11 de	11 b	L-III
Mojo Green	54 def	60 ab	53 a	36 b	36 d	35 c	12 cde	29 ab	E-IV
Gardensoy 42	58 def	n/a[†]	13 cd	n/a	68 ab	n/a	19 abcd	n/a	IV
Chiba Green	38 f	38 b	32 b	21 cd	56 c	62 ab	12 cde	17 ab	L-III
Mooncake	66 cde	78 a	19 c	16 cde	61 bc	49 bc	20 abc	35 a	V
BeSweet 2001	38 f	53 ab	7 d	5 e	66 ab	60 ab	27 a	35 a	III
Sayamusume	36 f	38 b	30 b	23 bc	58 bc	61 ab	11 cde	17 ab	III

[†]Gardensoy 42 was only evaluated in 2014. [‡] Maturity groups are estimated based on days to maturity (R6).

Table 4. Plant height and growth habit data recorded at R6 stage of development. Flower color was determined at first bloom. Within year and variable means followed by the same letter are not statistically different as determined via means separation at alpha= 0.05.

Variety	Plant Height		Height of first pod		Emergence percentage		Growth Habit[‡]	Flower color	First flower	
	cm				%				DAP	
	2014	2015	2014	2015	2014	2015			2014	2015
Owens	105 b	105 b	12 b	11 a	61 c	71 abc	Upright / Det.	White	51	54
Butterbean	66 f	66 f	8 cd	7 bc	82 ab	81 ab	Prostrate / Indet.	Purple	31	29
Lanco	80 cd	78 cd	7 cd	6 bc	79 abc	66 c	Upright / Det.	Purple	34	36
Gardensoy 51	88 c	82 c	9 c	5 c	82 ab	78 abc	Upright / Det.	White	36	36
Midori Giant	70 def	68 ef	6 cd	13 a	90 ab	76 abc	Upright / Det.	Purple	31	29
Mojo Green	79 cde	74 de	8 c	6 bc	89 ab	82 a	Upright / Det.	Purple	31	29
Gardensoy 42	80 cd	n/a[†]	9 c	n/a	78 abc	n/a	Upright / Det.	Purple	31	29
Chiba Green	65 f	56 g	8cd	6 bc	95 a	77 abc	Upright / Det.	White	31	29
Mooncake	168 a	155 a	16 a	8 b	81 ab	67 bc	Upright / Indet.	White	34	38
BeSweet 2001	63 f	45 h	5 d	3 d	74 bc	24 d	Upright / Det.	White	28	29
Sayamusume	68 ef	55 g	7 cd	6 bc	89 ab	76 abc	Upright / Det.	White	31	29

[†] Gardensoy 42 was only evaluated in 2014. [‡] Det. = determinate, Indet. = indeterminate

Table 5. Overall isoflavone concentration distribution (µg/g) as a function of cultivar and year. Average values were calculated from four replicate samples of each cultivar. †

	Isoflavone glycosides			Malonyl isoflavone			Acetyl isoflavones			Isoflavone aglycones			
Cultivar	daid	gly	gen	daid	gly	gen	daid	gly	gen	daid	gly	gen	Total
Mooncake													
2014	87.1a	48.2a	79.7a	102.7a	38.7a	138.9a	10.9a	0	0	1.38a	1.42ab	0.14a	509a
2015	4.81ab	2.3ab	7bc	104.5ab	29.7b	16.1c	0.34c	0	0	0a	0.18d	0	165c
Gardensoy 51													
2014	61.2ab	40.4ab	77.2ab	71.7ab	25.5b	132.6a	7.9ab	0	0	1.15ab	1.11bcd	0.07ab	419ab
2015	5.38a	4.5a	15.9a	118.4ab	29b	126.9a	3.6a	0	0	0a	0.5cbd	0	304a
Midori Giant													
2014	48.8abc	26.2cd	60.2abc	59.6abcd	22.3bc	89.9ab	3bc	0	0	0.42ab	0.40ef	0	311abc
2015	4.74ab	0.9b	5.2c	80.8bc	14.8cd	18.6c	0.73bc	0	0	0.19a	0.73ab	0	127cd
Butterbean													
2014	58.4ab	32bc	55abcd	64abc	21.4bcd	39b	1.9bc	0	0	0.50ab	0.65de	0b	273bcd
2015	4.2b	2.6ab	3.3c	43cd	7cd	5.3c	0.57bc	0	0	0.05a	0.29cd	0	66d
BeSweet 2001													
2014	36.5bc	24.8cd	41.8cd	57.4bcde	23.2bc	50.8b	4bc	0	0	0.57ab	0.79cde	0b	240bcd
2015	4.4b	3ab	5.1c	39cd	7.9cd	2.1c	1.28b	0	0	0a	0.78ab	0	63d
Lanco													
2014	33.2bc	31.3bc	47abcd	41.4bcde	29.5ab	42.8b	2.4bc	0	0	0.50ab	0.01f	0b	228cd
2015	4.6ab	3ab	5.3c	104.86ab	29.7b	32.6bc	0.75bc	0	0	0.14a	0.12d	0	181bc
Mojo Green													
2014	37.2bc	22.1cd	34.7cd	42.1bcde	18.4bcd	19.6b	0.8c	0	0	1.27ab	0.62de	0b	177cd
2015	4.6ab	1.7ab	5.4c	93ab	19.3bc	5.3c	0.2c	0	0	0.09a	0.17d	0	130cd
Owens													
2014	19.2c	29.8bc	33.8cd	22.9cde	20.9bcd	32b	1.5bc	0	0	0.22b	1.7ab	0b	162cd
2015	5ab	1.59ab	10.6b	132.3a	47.7a	49.7b	0.16c	0	0	0a	0.47bcd	0	248ab
Sayamusume													
2014	19c	16d	43.1bcd	23cde	10.15d	35.6b	3bc	0	0	0.25b	1.27abc	0b	151cd
2015	4.26b	1.3b	3c	30.2d	5.6d	5.9c	0.67bc	0	0	0.12a	0.71abc	0	52d
Chiba Green													
2014	14.3c	16.2d	40.1cd	20.2de	9.9d	27.6b	2.9bc	0	0	0.28b	1.71a	0b	133cd
2015	4.7ab	1.3	4.5c	69.8bcd	7.1cd	10.9c	0.8bc	0	0	0.14a	1.01a	0	100cd
Gardensoy 42													
2014	14.8c	16.4d	22.2d	14.6e	12.1cd	23b	1.2c	0	0	0.37ab	0.48ef	0b	105d
2015	* ‡	*	*	*	*	*	*	*	*	*	*	*	*

† Means from the same year and column followed by the same letter are not statistically different as determined by means separation at α = 0.05. Daid= daidzien, gly= glycitein, gen= genistein.

‡ Gardensoy 42 was only evaluated in 2014.

Table 6. Climate data for each growing season (May-Sept) as recorded by the on-site weather station located at E. V. Smith Research Unit in Shorter, Alabama.

	Year	
	2014	2015
Average air temperature (C)	25.0	25.7
Average soil temperature (C, at10 cm)	28.2	27.3
Highest average temp during R5 (C)	32.9	34.1
Evapotranspiration (ET mm/day)	0.21	0.23
Rainfall total (cm)	33.6	32.8
Day length at seeding (hours)	14.1	14.3

Table 7. Correlation coefficients and significance of total isoflavone content as a function of year and temperature at α 0.05.

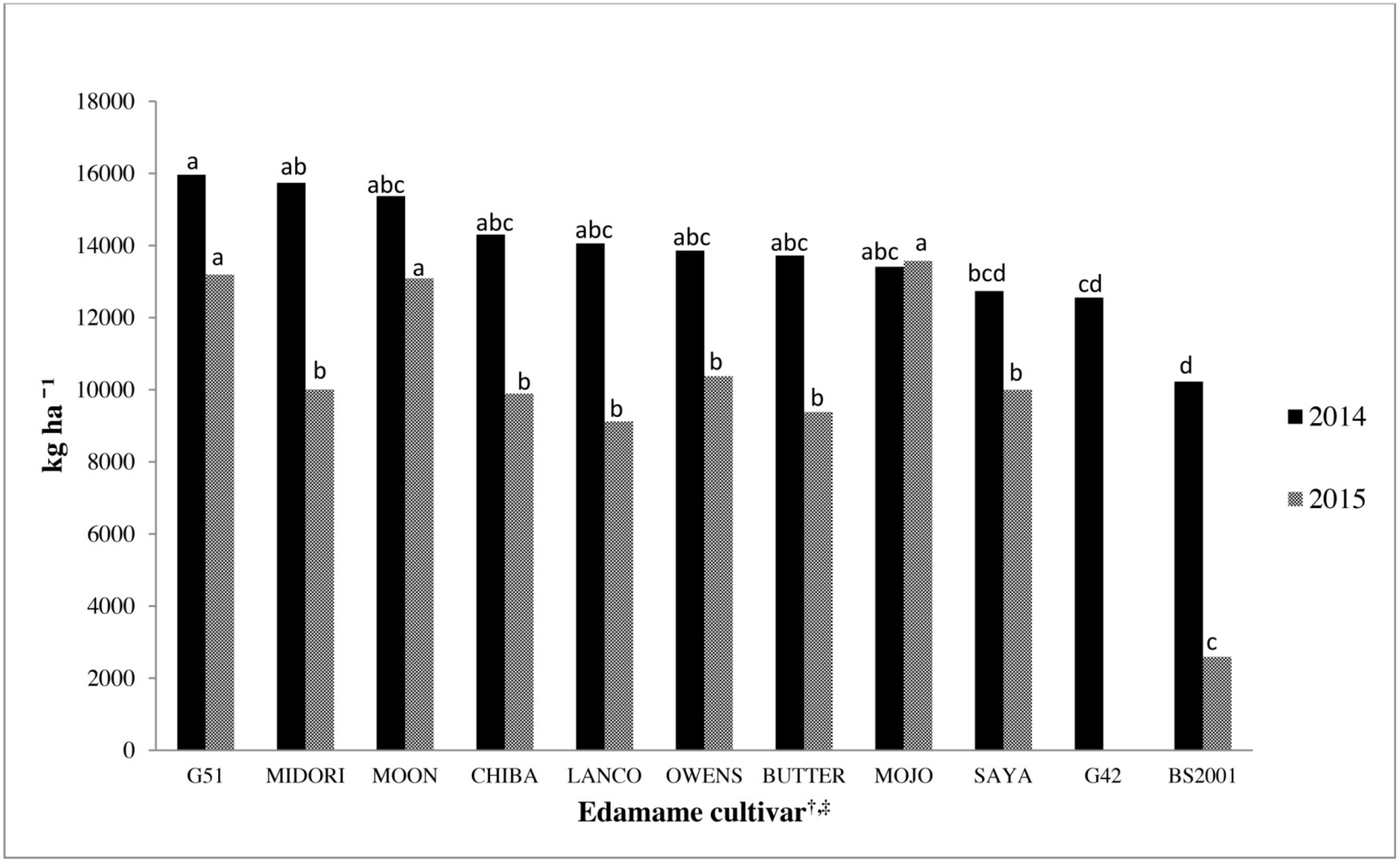
Total isoflavone content (µg/g)	Correlation coefficients with p values		
	High temperature	**Low temperature**	**Average temperature**
2014	0.34 ($p = 0.02$)	-0.008 ($p = 0.95$)	0.23 ($p = 0.13$)
2015	-0.71 ($p < 0.0001$)	-0.61 ($p < 0.0001$)	-0.67 ($p < 0.0001$)

Table 8. Plant height, emergence, and leaf K. Means within a year and column followed by the same letter are not statistically different as determined via means separation at alpha= 0.05.

Rate of Potassium (kg ha-1)	**Plant Height (cm)†**			**Emergence percentage‡**						**Leaf K %‡**					
	Chiba	Midori	Owens	Chiba		Midori		Owens		Chiba		Midori		Owens	
	cm			**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
0	46.9a	56.9a	55.4a	71.9a	93.1a	72.2a	88.1a	80.6a	72.5a	1.7a	1.9a	1.8a	1.9a	2.2a	1.7b
67	48.4a	56.4a	54.5a	65.9a	95.3a	81.6a	85.9a	71.6a	73.4a	1.7a	2.0a	1.8a	2.0a	2.4a	2.0ab
100	49.5a	60.2a	55.3a	62.5a	90.6a	76.6a	82.5a	77.8a	69.4a	1.6a	2.0a	1.8a	2.1a	2.4a	2.2ab
134	47.3a	56.9a	55.5a	60.9a	87.2a	75.9a	77.8a	77.2a	67.2a	1.6a	2.1a	1.8a	2.2a	2.4a	2.3a

† Plant height data was not significantly affected by year and were therefore combined into one dataset. ‡ Data was separated by year due to a significant interaction between cultivar and year.

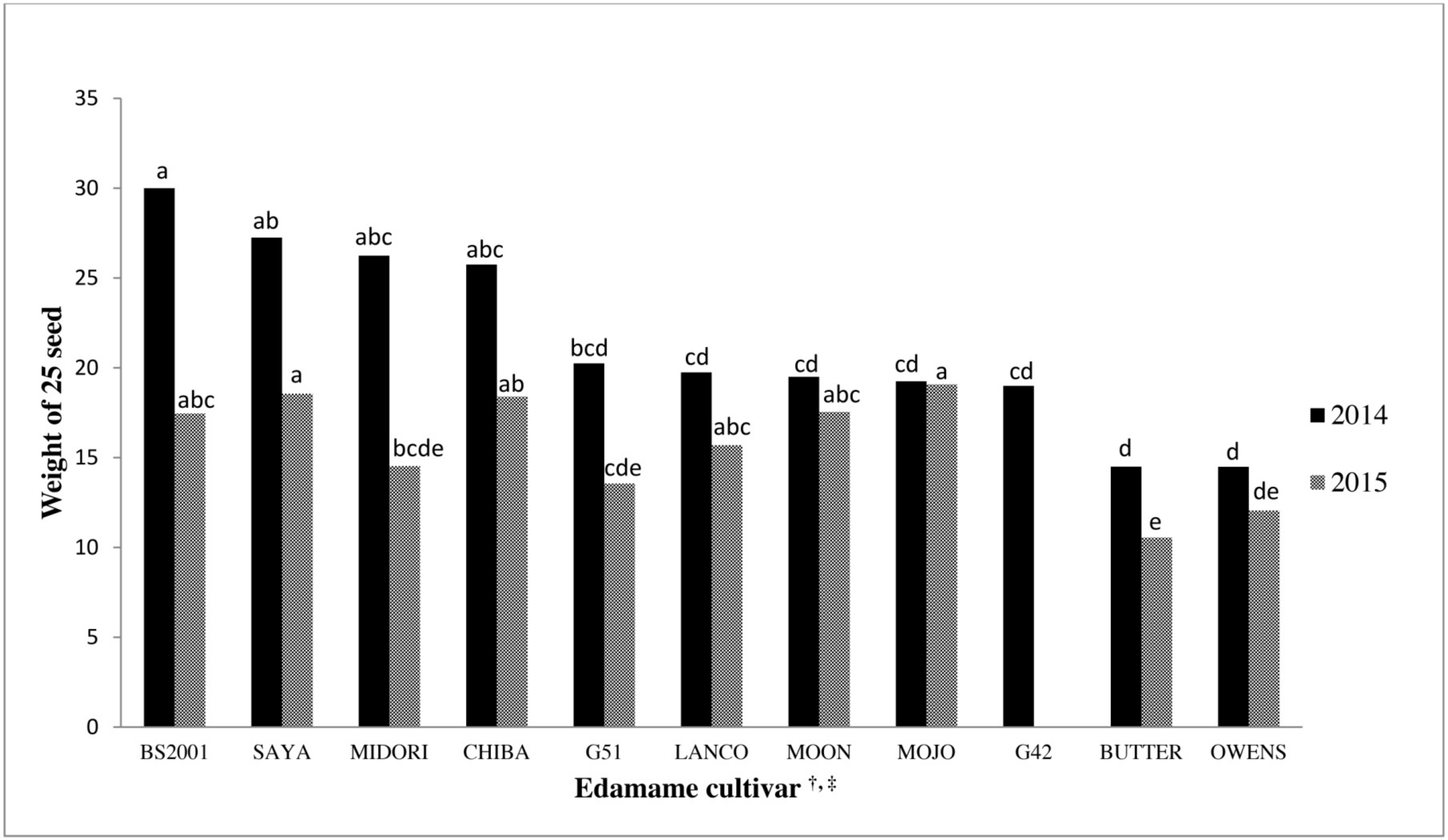
Fig. 1. Yield of mature green pods as effected by edamame cultivar, Tallassee, AL. Within each year, columns with the same letter are not statistically different as determined via means separation at alpha = 0.05.



† Chiba = Chiba Green, Mojo = Mojo Green, Saya= Sayamusume, Midori= Midori Giant, G51= Gardensoy 51, Butter = Butterbean, Moon = Mooncake, G42 = Gardensoy 42, and BS2001 = BeSweet 2001.

‡ Gardensoy 42 was only evaluated in 2014.

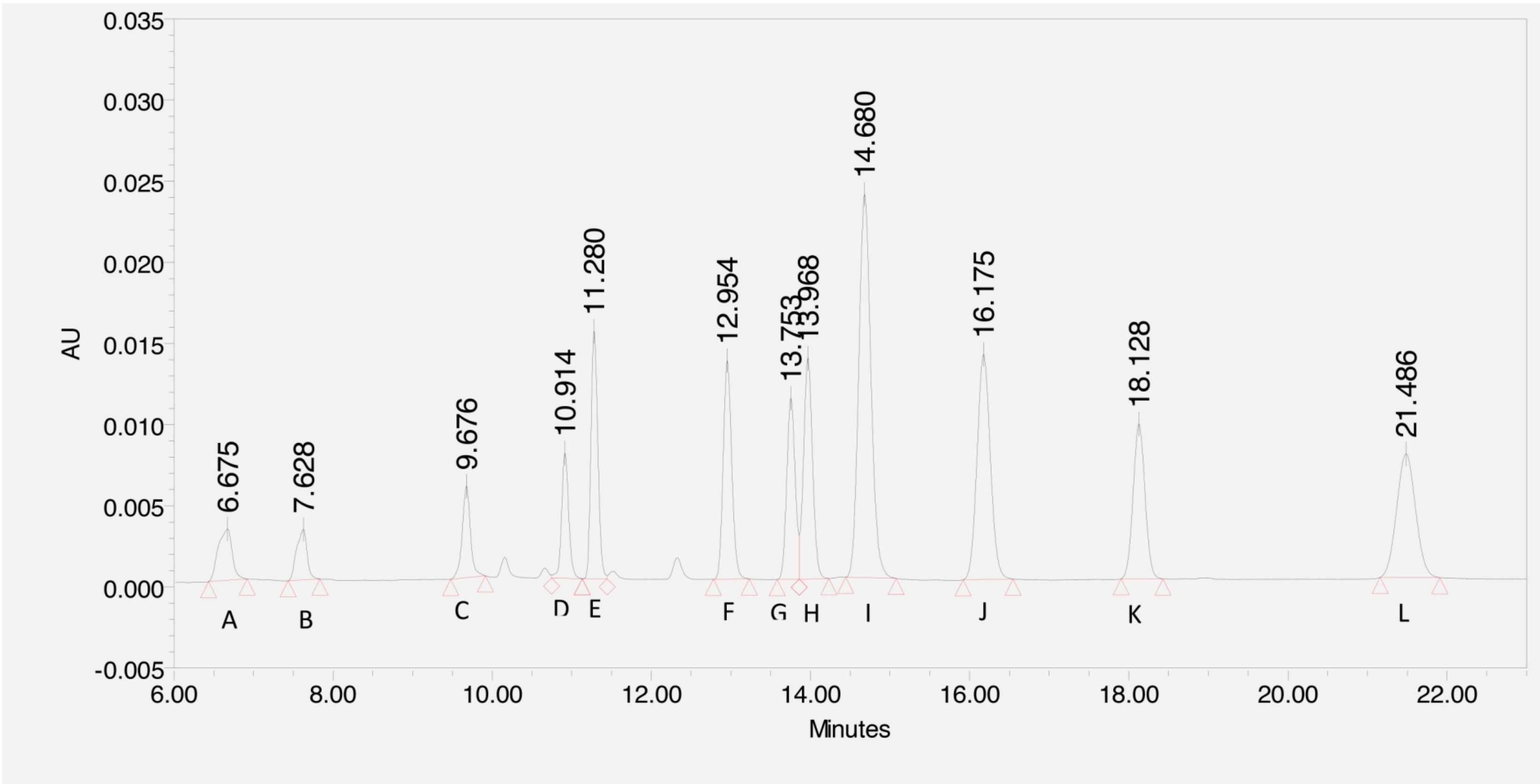
Fig. 2. Weight (g) of a 25 bean sample collected from a five plant sub-sample at harvest. Within each year, column with the same letter are not statistically different as determined via means separation at alpha= 0.05.



† Chiba = Chiba Green, Mojo = Mojo Green, Saya= Sayamusume, Midori= Midori Giant, G51= Gardensoy 51, Butter = Butterbean, Moon = Mooncake, G42 = Gardensoy 42, and BS2001 = BeSweet 2001.

‡ Gardensoy 42 was only evaluated in 2014.

Figure 3. Chromatogram of 12 isoflavone standards using a Waters Acquity UPLC system equipped with PDA detector (210-280 nm). An Acquity UPLC BEH C18 column (2.1 X 50 mm, 1.7 um particle size) was used for separation.



Legend:

A – Daidzin, B – Glycitin, C – Genistin, D – 6"-O- Malonyl daidzin, E - 6"-O- Malonyl glycitin, F – 6"-O- Acetyl daidzin, G - 6"-O- Acetyl glycitin, H - 6"-O- Malonyl genistin, I – Daidzein, J – Glycitein, H -6"-O- Acetyl genistin, L – Genistein.

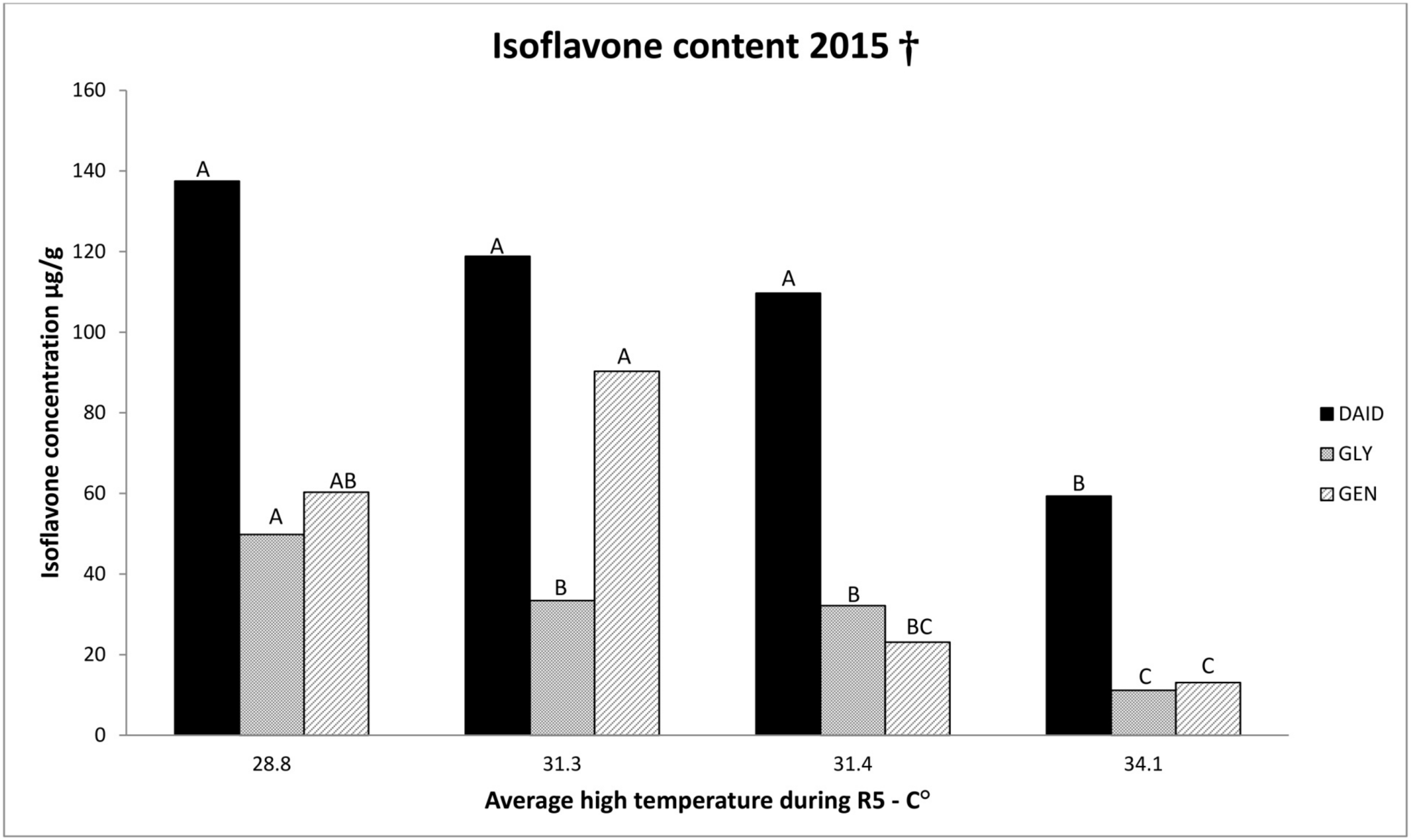


Fig. 4. Mean content of daidzein (Daid), glycitein (Gly), and genistein (Gen) and their conjugants as a function of temperature during the R5 stage of seed development in the 2015 growing season.

† Columns of the same color that share a common letter are not statistically different at α 0.05.

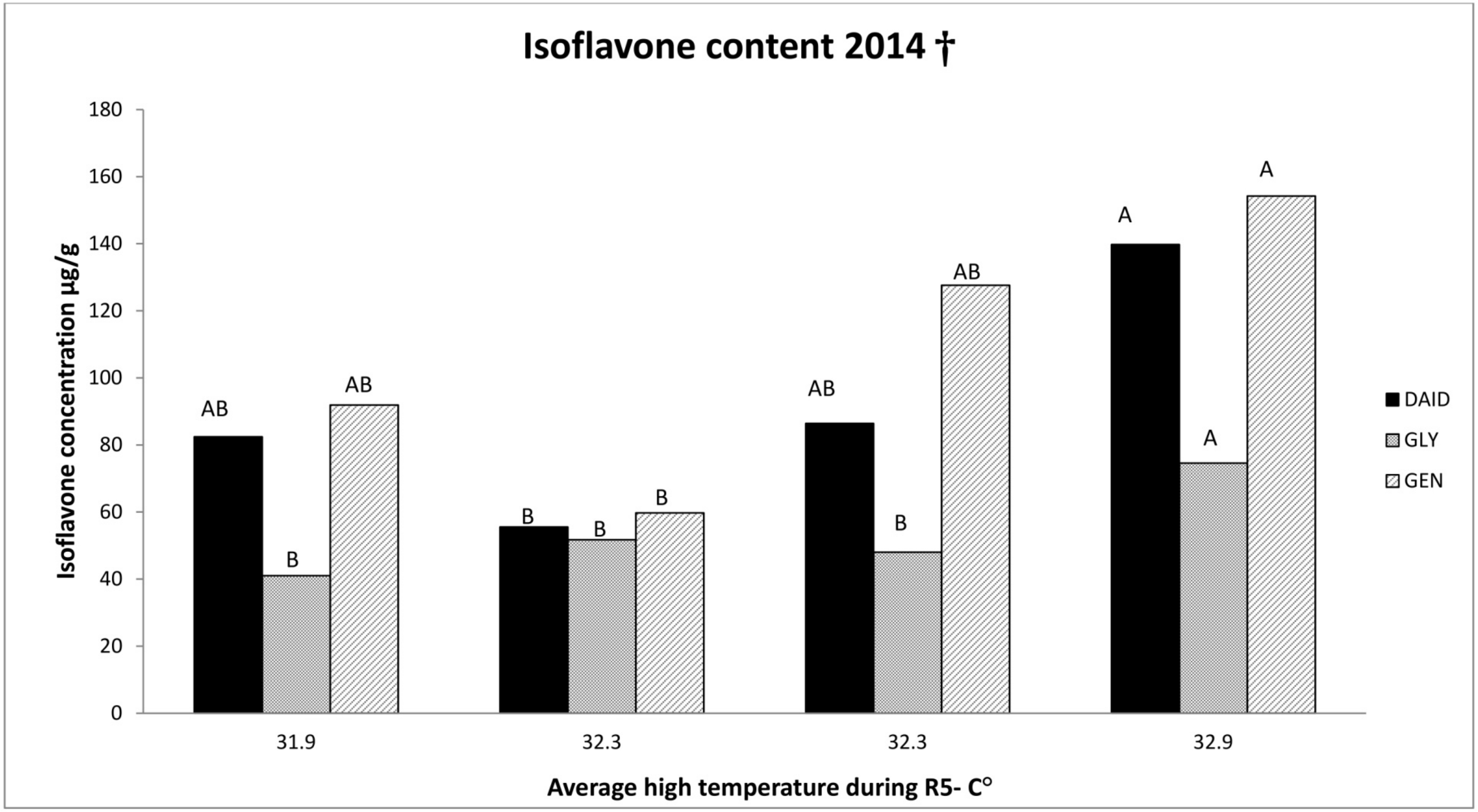


Fig. 5. Mean content of daidzein (Daid), glycitein (Gly), and genistein(Gen) and their conjugants as a function of temperature during the R5 stage of seed development in the 2014 growing season.

† Columns of the same color that share a common letter are not statistically different at α =0.05.

Fig. 6. Image of labeled trays used in sensory evaluations and the Latin square used to ensure a counter balanced design.

	1	2	3	4	5	6
A	Madori Giant	Mojo	Saya	Chiba	Owens	G51
B	Mojo	Saya	Chiba	Owens	G51	Madori Giant
C	Saya	Chiba	Owens	G51	Madori Giant	Mojo
D	Chiba	Owens	G51	Madori Giant	Mojo	Saya
E	Owens	G51	Madori Giant	Mojo	Saya	Chiba
F	G51	Madori Giant	Mojo	Saya	Chiba	Owens

Fig. 7. Overall likeness of six commercially available edamame cultivars collected at four sensory evaluations during the 2015 and 2016 growing seasons. Columns with the same letter are not statistically different at α = 0.05.

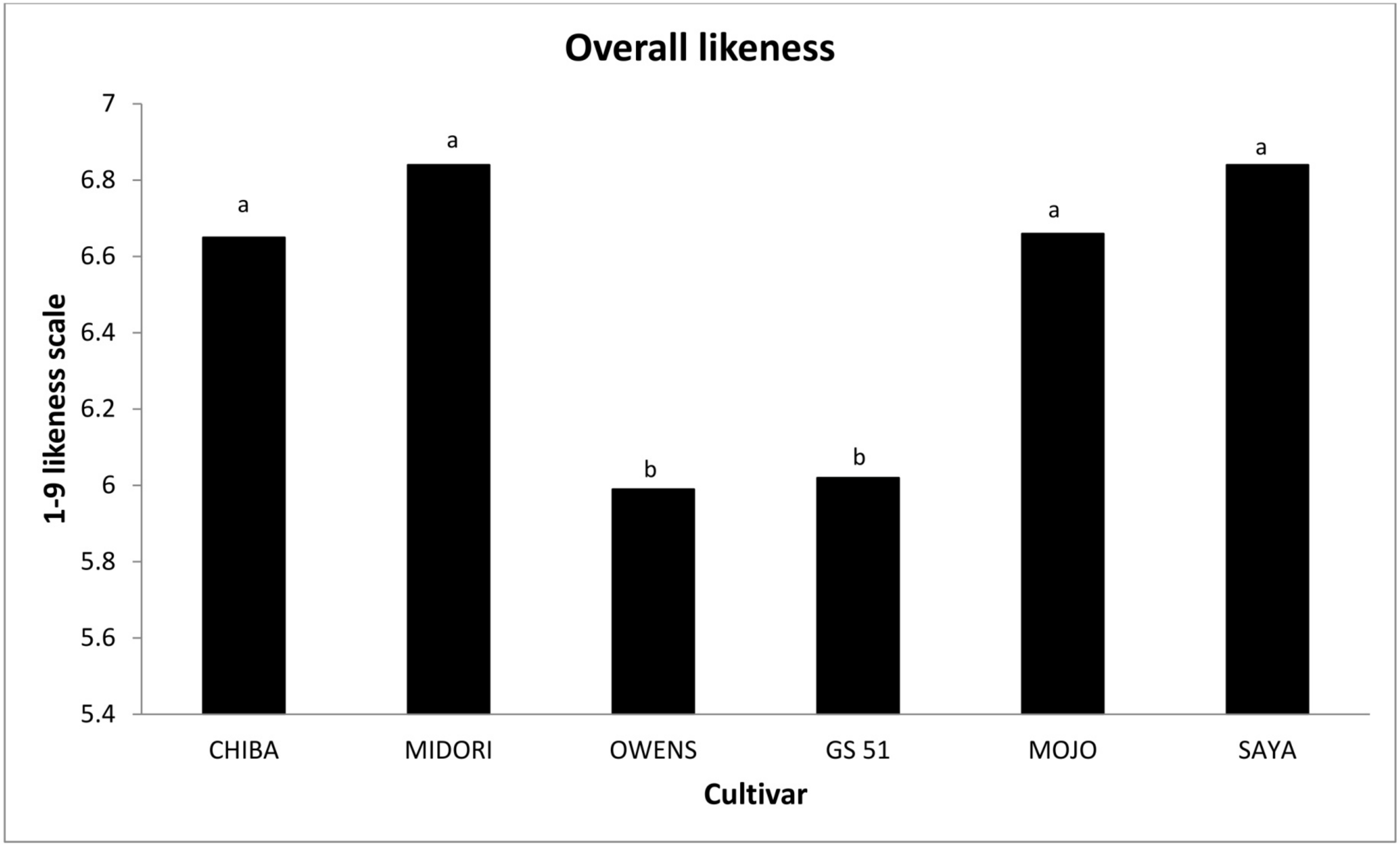


Fig. 8. Likeness of external appearance of six commercially available edamame cultivars collected at four sensory evaluations during the 2015 and 2016 growing seasons. Columns with the same letter are not statistically different at $\alpha = 0.05$.

Fig. 9. Likelihood to purchase edamame after completeing the sensory evaluation study during the 2015 and 2016 growing season.

VL = very likely, SL= somewhat likely, N= neutral, and U= somewhat unlikely or unlikely.

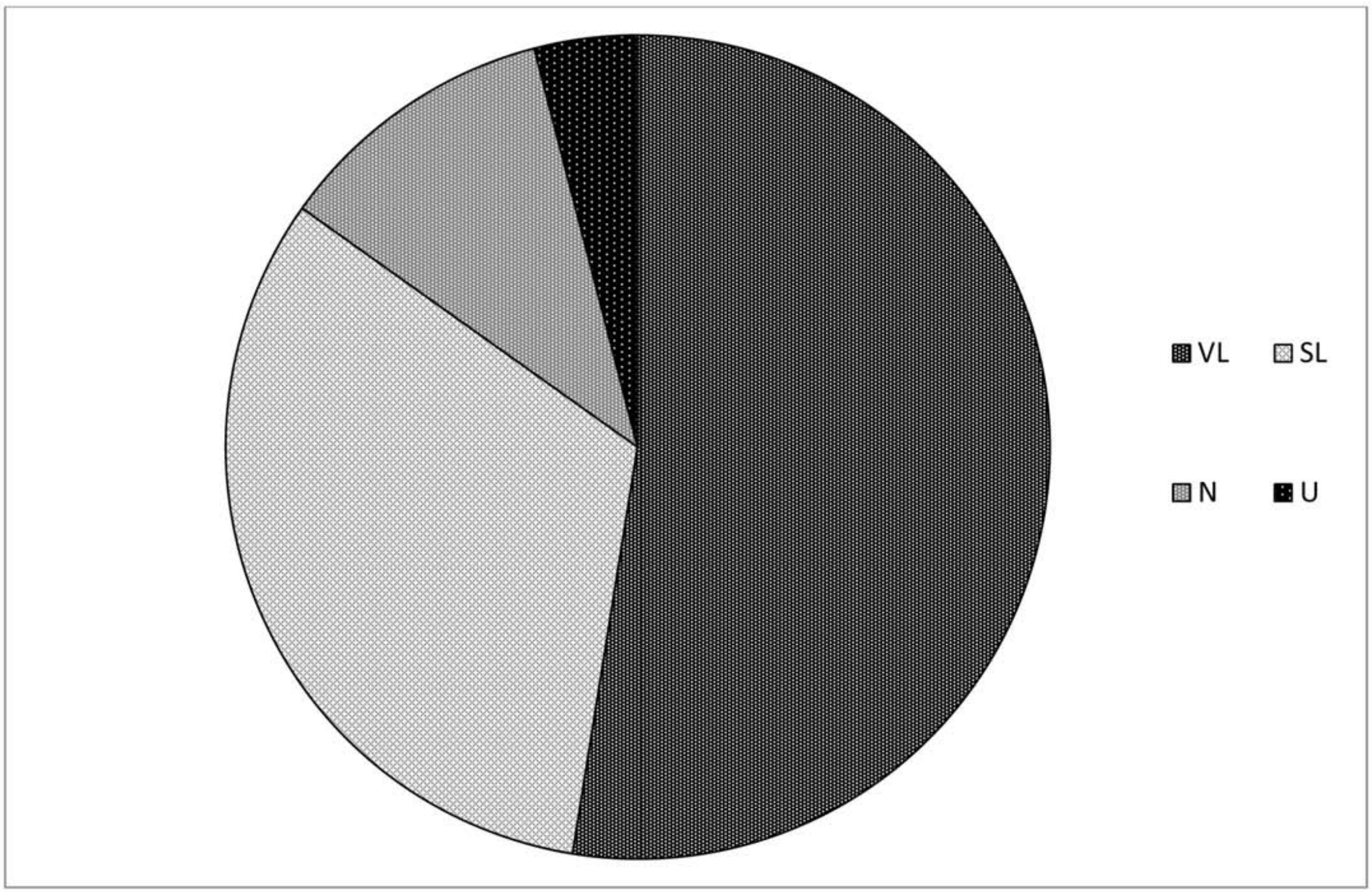
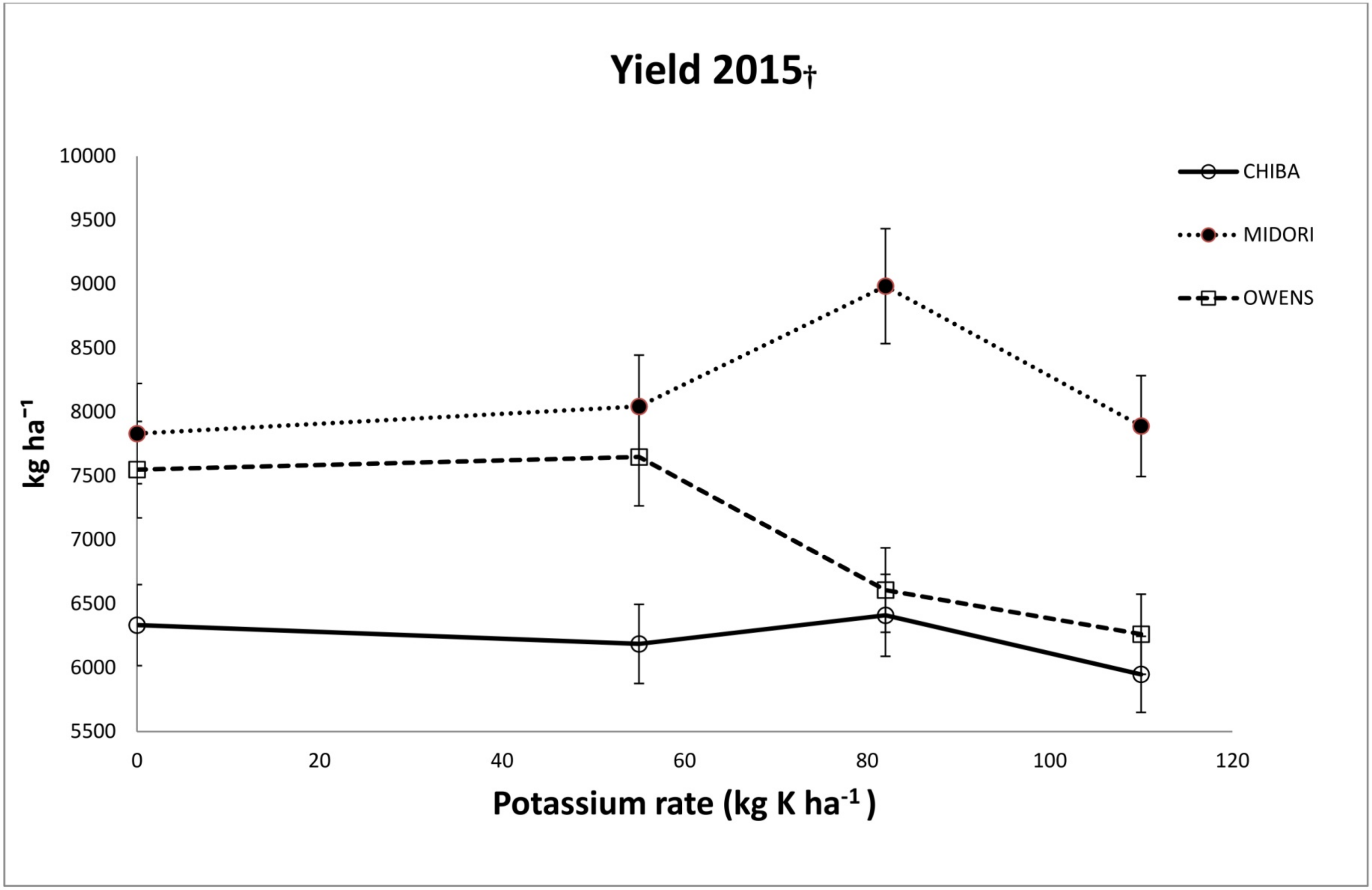
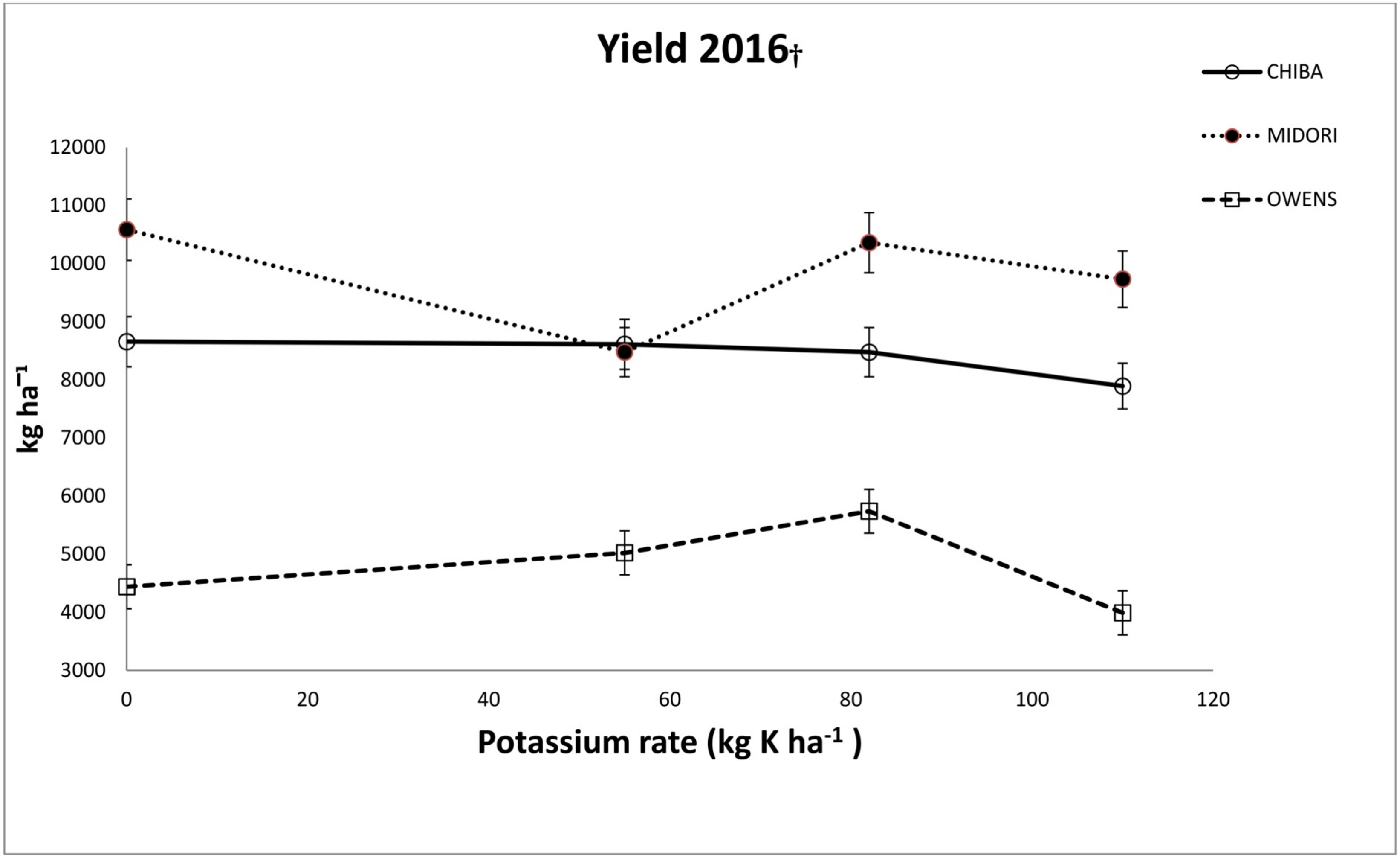


Fig 10. Marketable yield of mature green edamame pods as affected by cultivar and potassium (K) rates for the 2015 growing season, Tallassee, AL.



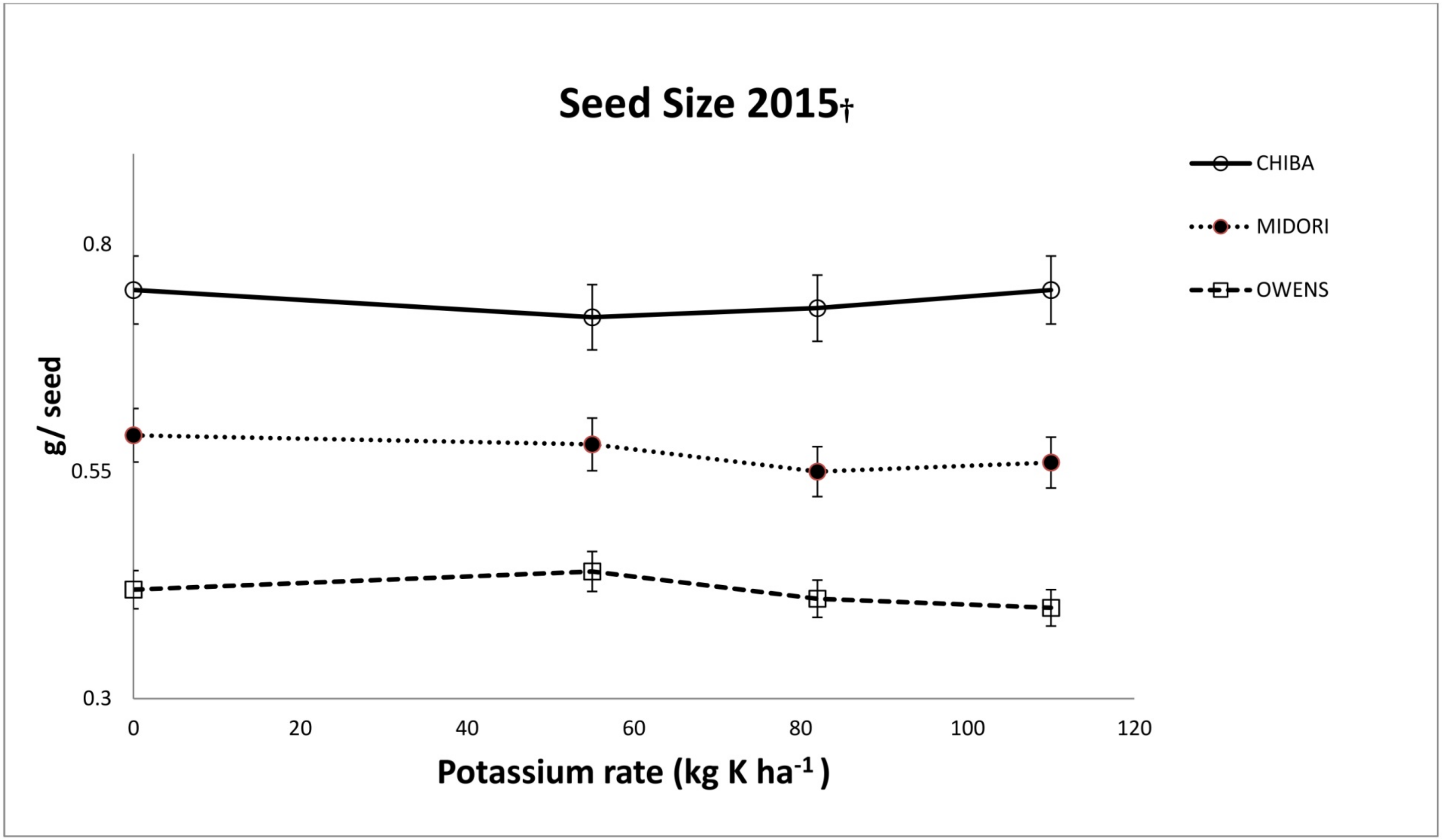
† Chiba = Chiba Green, Midori = Midori Giant, and Owens = Owens.

Fig 11. Marketable yield of mature green edamame pods as affected by cultivar and potassium (K) rates for the 2016 growing season, Tallassee, AL.



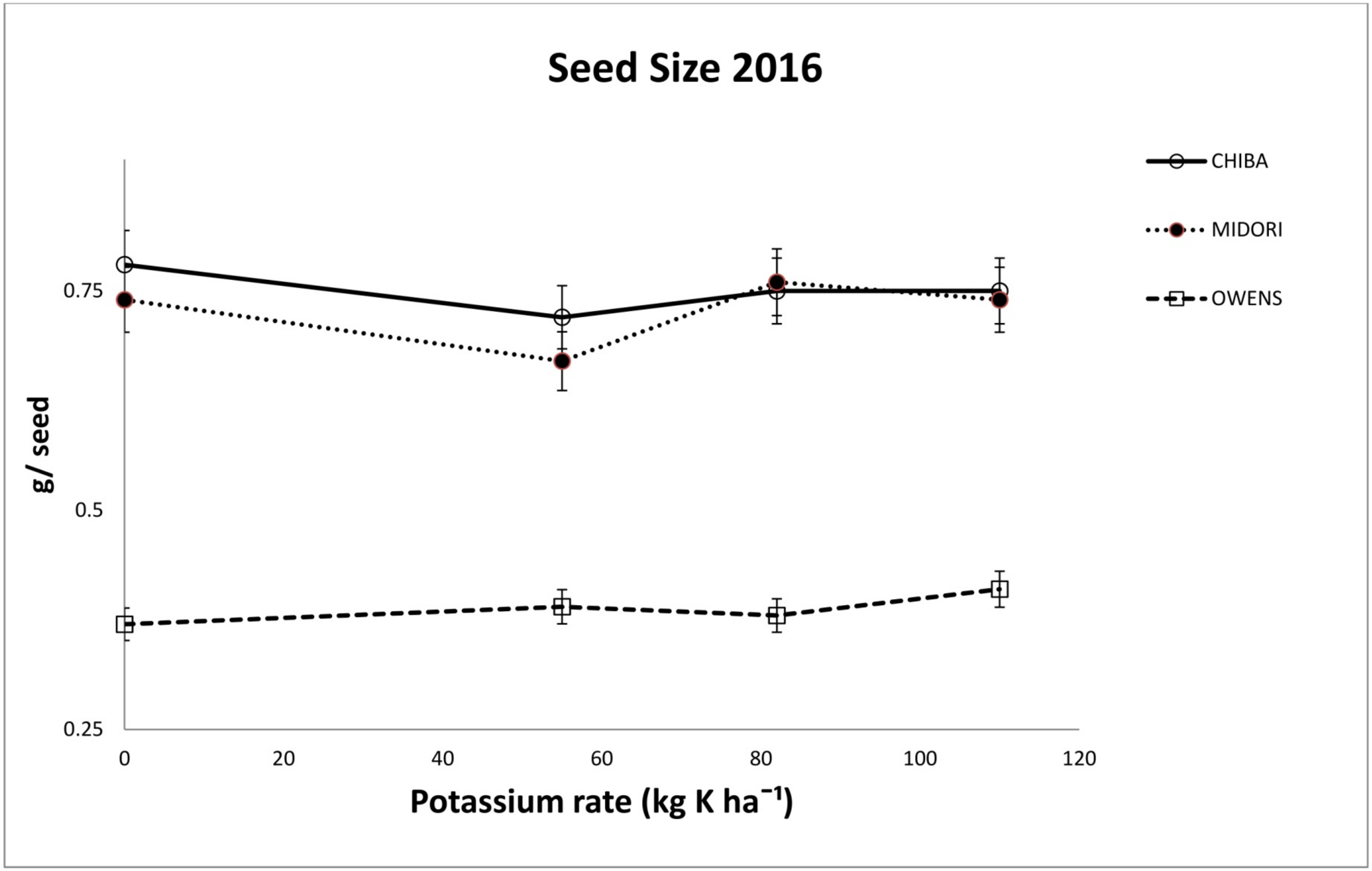
† Chiba = Chiba Green, Midori = Midori Giant, and Owens = Owens.

Fig 12. Seed size of mature green edamame as affected by cultivar and potassium (K) rates for the 2015 growing season, Tallassee, AL.



† Chiba = Chiba Green, Midori = Midori Giant, and Owens = Owens.

Fig 13. Seed size of mature green edamame as affected by cultivar and potassium (K_20) rates for the 2016 growing season, Tallassee, AL.



† Chiba = Chiba Green, Midori = Midori Giant, and Owens = Owens.