RESEARCH

Phenomorphological Characterization of Vegetable Soybean Germplasm Lines for Commercial Production

Martin M. Williams II*

ABSTRACT

Growing demand for vegetable soybean [*Glycine max* (L.) Merr.] has renewed interest in producing the crop in the United States, a significant importer of vegetable soybean despite being the world's largest producer and exporter of grain-type soybean. Field studies were conducted over 3 yr to(i) compare phenomorphological traits of vegetable and grain-type soybean and (ii) identify candidate lines for vegetable soybean production in the North Central United States, the nation's leading soybean-producing region. A total of 136 vegetable soybean entries from 22 sources were compared to 14 grain-type cultivars representing a range of maturity groups. Germination and emergence of vegetable soybean were poorer than grain-type entries. Seedling growth traits and rate of phenological development were higher in vegetable soybean. However, by the time of crop harvest (i.e., R6 growth stage), vegetable soybean produced shorter, smaller plants than grain-type soybean. Seed mass accounted for some of the variation in emergence and seedling traits. Filtering entry responses by criteria essential to viable commercial production, including a sensory evaluation, 12 entries from eight seed sources were identified as the most promising candidate lines for use in the North Central United States. By comparing vegetable soybean responses to grain-type soybean, this work puts into perspective the agronomic performance of vegetable soybean germplasm available to growers in the United States and points to specific areas of future research and crop development.

USDA-ARS, Global Change and Photosynthesis Research Unit, Univ. of Illinois, Urbana, IL 61801. Received 6 Oct. 2014. Accepted 13 Dec. 2014. *Corresponding author (mmwillms@illinois.edu).

Abbreviations: MG, maturity group; WAP, weeks after planting.

Demand for vegetable soybean, a nutraceutical food-grade soybean also known as "edamame", has been rising in the United States as well as other parts of the world. Historically a food of East Asian cultures, vegetable soybean now can be found in various supermarkets and chain restaurants throughout the United States. Between the years 2000 and 2008, Sams et al. (2012) reported a fourfold increase in consumption of vegetable soybean in the United States. A majority of the vegetable soybean consumed in the United States is imported (Mebrahtu and Mullins, 2007), primarily from China, the largest producer, consumer, and exporter of vegetable soybean (Dong et al., 2014).

The United States leads global production and export of grain-type soybean and has one of the most productive soybean-growing regions of the world. Soybean is grown on ~30 million hectare each year, valued in excess of $40 billion (NASS, 2014). The top soybean-producing states are located in the North Central United States and include Illinois, Iowa, Indiana, Minnesota, Nebraska, Ohio, Missouri, and South Dakota (NASS, 2014). Conceivably, environments favorable for grain-type soybean also would be well suited for vegetable soybean production. In fact, producing vegetable soybean in the United States is not a new idea. Several state agricultural experiment stations tested cultivars from China, Korea, and Japan in the early 20th century (Morse, 1930). Since then, examples of sustained vegetable soybean production in the

Published in Crop Sci. 55:1274–1279 (2015).
doi: 10.2135/cropsci2014.10.0690

United States have been limited. Perhaps due in part to growing consumer interest in the crop, and in domestically grown products in particular, several vegetable processors in the United States have initiated pilot production with aims to expand to a commercial level.

Vegetable soybean is differentiated from grain-type soybean in several ways. Vegetable soybean is harvested at an immature seed stage (i.e., R6) as opposed to a dry seed state (i.e., R8) for grain-type soybean (crop growth stages based on Fehr and Caviness, 1977). Vegetable soybean cultivars produce large seeds with a sweet, nutty, and mild flavor. While some vegetable soybean cultivars have been developed for U.S. production systems, achieving a high-quality product from the narrow genetic diversity observed in available germplasm has been a challenge (Mimura et al., 2007). For instance, the number of vegetable soybean lines available for domestic production, from both commercial and public sources, is likely <200 and the yield potential of many of these lines may limit their commercial usefulness. Exactly which cultivars not only grow well in the North Central United States but also are suitable for vegetable processing is poorly known.

Although vegetable soybean cultivars are unique, agronomic practices for the crop are largely borrowed from grain-type soybean production. Variety trials, ranging from four to eight cultivars, have been conducted at a few locations around the United States in recent years (Carson et al., 2011; Duppong and Hatterman-Valenti, 2005; Sánchez et al., 2005; Zhang and Kyei-Boahen 2007). Generally, field trials are managed similarly to grain-type soybean, including planting date, planting equipment, seeding rates, fertility management, and irrigation. Certain agronomic practices are believed to influence flavor of vegetable soybean (Duppong and Hatterman-Valenti, 2005). Few pesticides are registered for use on vegetable soybean; those registered on the crop have been used in grain-type soybean for years (Williams and Nelson, 2014). In order for sustained domestic production to be realized, cultivation practices will be tailored to the unique aspects of vegetable soybean. In the meantime, an understanding of how vegetable soybean growth and development compares to grain-type soybean might identify specific improvements needed in vegetable soybean cultivation and germplasm development. The objectives of the study were to (i) compare phenomorphological traits of vegetable and grain-type soybean and (ii) identify candidate lines for vegetable soybean production in the North Central United States.

MATERIALS AND METHODS

Germplasm

Seed of vegetable soybean entries were procured from 22 sources, representing most commercial and public cultivars available in the United States. Sources included four university vegetable soybean breeding programs (Iowa State Univ., Univ. of Arkansas, Univ. of Illinois, and Washington State Univ.). In addition, other vegetable soybean entries were selected from the USDA Soybean Germplasm Collection. Criteria used to select entries from the collection included vegetable soybean, parental lines of U.S.-developed vegetable soybean, and large-seeded entries introduced from other countries with names associated with vegetable soybean. A total of 136 vegetable soybean entries were obtained (Supplemental Table S1). Fourteen grain-type soybean entries were included each year. Grain-type entries included lines adapted to the North Central United States, representing a range of maturity groups (i.e., MG 00–MG VI), as well as some lines with known tolerance or sensitivity to bentazon herbicide. To use seed from the same maternal environment in all but the first year, seed from experiments was collected after maturity and used the following spring.

Experimental Approach

Field experiments were conducted at the University of Illinois Vegetable Crop Farm near Urbana. The soil was a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) averaging 35 g kg^{-1} organic matter and a pH of 5.8. Experiments followed the soybean year of a sweet corn (*Zea mays* L.)–soybean rotation. Before planting, fields received two passes of a field cultivator.

The experimental design was a randomized complete block with three replications. An experimental unit was a single 2.5 m length of row planted with 50 seeds. A cone planter set on 76-cm row spacing was used to plant trials on 3 June, 18 May, and 22 May of 2011, 2012, and 2013, respectively. Planting depth was 3.0 cm. Immediately after planting, 1.8 kg metolachlor ha^{-1} was applied for preemergence weed control. Emerged weeds were removed by hand-hoeing and interrow cultivation, as necessary. Experiments were sprinkler irrigated as needed to avoid drought conditions.

Data Collection

Fourteen phenomorphological traits were quantified (Table 1). Seed germination of all entries was quantified before planting using a rolled towel test at 21°C (Baalbaki et al., 2009). Stand counts were made 3 weeks after planting (WAP) to determine emergence, based on the percentage of seed planted. Plant height was measured from the soil surface to plant apex on three plants per plot at 3 WAP, 6 WAP, and at the R6 stage. Once the first trifoliate leaf was fully expanded (3 WAP), one trifoliate leaf per plot was clipped at the petiole base, analyzed with an area meter (LI-3100C, LI-COR, Lincoln, NE), dried to constant mass, and weighed. When the first trifoliate leaf was harvested, the relative size of the second trifoliate leaf was recorded (0 = none to 10 = comparable to first trifoliate leaf). Whole-plant leaf area (WLA) was calculated using the following formula:

$$WLA = LA_1 + (RS_2/10 \times LA_1) \quad [1]$$

where LA_1 = leaf area of first trifoliate, RS_2 = relative size of second trifoliate. Canopy area 6 WAP and at R6 was calculated as the product of plant height and width averaged over three plants per plot. Days from planting to R1 (i.e., “beginning flowering”) and R6 were recorded. At maturity, seed mass was quantified and occurrence of shattering was noted.

Table 1. Phenomorphological traits of grain-type and vegetable soybean entries in field trials conducted in 2011, 2012, and 2013 near Urbana, IL.

Trait	Unit	Description
Germination	%	germination observed in rolled towel tests
Emergence	%	field emergence, 3 WAP
Height 3WAP†	cm	plant height from soil surface to plant apex
Trifoliate biomass 3WAP	g	mass of first trifoliate, fully expanded
Trifoliate leaf area 3WAP	cm^2	leaf area of first trifoliate, fully expanded
Whole-plant leaf area 3WAP	cm^2	leaf area of whole plant
Height 6WAP	cm	plant height from soil surface to plant apex
Canopy area 6WAP	cm^2	plant height multiplied by plant width
Time to R1	days	days from planting to R1, beginning flowering
Time to R6	days	days from planting to R6, full seed
Height R6	cm	plant height from soil surface to plant apex
Canopy area R6	cm^2	plant height multiplied by plant width
Seed mass	g 100 $seed^{-1}$	dry mass of 100 seed, R8
Shattering	–	0 = no shattering at full maturity or 1 = shattering, at R8

† WAP, weeks after planting.

Data Analysis

The null hypothesis tested in objective 1 was that phenomorphological traits were comparable between vegetable soybean and grain-type entries. Response variables between grain-type and vegetable soybean entries were characterized by unequal sample sizes and variances. Moreover, variances were found to be non-homogeneous among years. Therefore, the Kolmogorov–Smirnov test of frequency distributions was used to determine if phenomorphological traits differed between grain-type and vegetable soybean entries each year. Analyses were conducted in SYSTAT 13 and hypotheses were tested at $\alpha = 0.05$.

Objective 2 was addressed by comparing performance of individual vegetable soybean entries against criteria essential to viable commercial production. Specifically, the database of responses of 136 vegetable soybean entries was used to identify entries with above-average field emergence, did not exceed a maximum height suitable for machine harvest, produced large seed suitable for the U.S. market, and passed a sensory evaluation of pods and seeds. Thresholds for plant height and seed mass were based on published literature. The sensory evaluation of fresh pods and seeds, collected at R6, was conducted by a vegetable processor, and included an assessment of pod size, pod color, seed color, seed blemishes, seed texture, and flavor; characteristics important to consumers (Wszelaki et al., 2005). Criteria were used to filter mean responses of each entry to identify candidate lines for vegetable soybean production in the North Central United States.

RESULTS AND DISCUSSION

Phenomorphological Traits

Seedling establishment was poorer in vegetable soybean than grain-type entries. For example, mean emergence of vegetable soybean was 11 to 24% lower than mean emergence of grain-type soybean (Table 2). Relative to grain-type entries, reduced emergence in vegetable soybean appeared to be due in part to lower germination also observed in vegetable soybean. However, germination alone did not explain poor emergence in vegetable soybean, since mean germination exceeded 78%, while mean emergence was below 35%. Adequate crop establishment has proven difficult in several environments (Duppong and Hatterman-Valenti, 2005; Rao et al., 2002; Sánchez et al., 2005). Clearly, methods to improve crop establishment will be critical to successful vegetable soybean production (Zhang et al., 2013). Preliminary results indicate soil pathogens contribute, in part, to low vegetable soybean emergence, as evidenced by specific fungicide seed treatments improving field emergence (M. Williams and C. Bradley, unpublished data, 2014).

Seedlings of vegetable soybean were larger than grain-type soybean. For example, at 3 WAP, vegetable soybean seedlings were taller than grain-type soybean and had larger, denser first trifoliate leaves (Table 2). Whole-plant leaf area at 3 WAP was highest in vegetable soybean entries in most years. Difference in seedling size between soybean types was in part the result of seed size used at planting, as vegetable soybean entries in this work produced larger seed than grain-type soybean. For example, mean seed mass of vegetable soybean entries was 55 to 76% higher than grain-type entries (Table 2). Previous researchers have found positive associations between soybean seed size and embryo size (Burris et al., 1971), seedling height (Burris et al., 1973), cotyledon leaf area (Burris et al., 1973), ground cover (Place et al., 2011a), and greater root and shoot mass (Longer et al., 1986). Place et al. (2011b) found that soybean seed size was the most influential trait on the crop's ability to suppress weeds. Limited herbicide registration and poor weed control are major impediments to domestic production of vegetable soybean (Williams and Nelson, 2014). While seed size will not become a stand-alone weed control tactic, the extent to which accelerated seedling growth contributes to competitive suppression of weeds merits further study.

Although large seed size of vegetable soybean apparently favors seedling growth, does it also come at a cost to crop emergence? No research has been conducted in vegetable soybean; however, work on grain-type soybean may shed some light. Large-seed size benefits to soybean vigor and growth observed by others has generally been for cultivars with seed mass <20 g 100 $seed^{-1}$ (Burris et al., 1971; Place et al., 2011a); considerably smaller seed than most vegetable soybean lines. Previous studies have

Table 2. Mean response of grain-type (grain) and vegetable (veg) soybean entries in field trials conducted in 2011, 2012, and 2013 near Urbana, IL. Differences in soybean types (P) were determined by the Kolmogorov–Smirnov test of frequency distributions.

Trait	Unit	2011			2012			2013		
		Grain	Veg	P	Grain	Veg	P	Grain	Veg	P
Germination	%	-[†]	–	–	92.1	83.9	<0.001	88.4	78.4	0.001
Emergence	%	–	–	–	57.5	33.0	<0.001	46.1	34.9	0.004
Height 3WAP[‡]	cm	7.5	8.7	<0.001	9.8	10.6	0.033	11.6	12.3	0.009
Trifoliate biomass 3WAP	g	0.128	0.153	<0.001	0.131	0.148	0.041	0.126	0.165	<0.001
Trifoliate leaf area 3WAP	cm^2	31.9	38.2	<0.001	25.1	28.4	0.021	25.0	32.7	0.001
Whole-plant leaf area 3WAP	cm^2	43.9	53.4	<0.001	55.8	63.8	0.090	41.6	58.2	<0.001
Height 6WAP	cm	37.5	38.5	0.151	30.2	29.5	0.386	44.1	42.4	0.756
Canopy area 6WAP	cm^2	1520	1550	0.337	1100	1050	0.534	1900	1860	0.716
Time to R1	days	52.1	45.1	<0.001	58.8	52.0	<0.001	59.3	53.1	0.030
Time to R6	days	86.6	77.1	<0.001	111.4	93.5	<0.001	104.8	90.7	0.008
Height R6	cm	-[§]	–	–	101.1	70.7	<0.001	88.9	63.2	<0.001
Canopy area R6	cm^2	–	–	–	8510	4600	<0.001	6080	3930	<0.001
Seed mass	g 100 $seed^{-1}$	13.6	22.9	<0.001	14.9	23.1	<0.001	13.9	24.4	<0.001
Shattering	–	–	–	–	0.143	0.629	0.004	0.143	0.637	0.003

[†] Data in 2011 were not included due to potential influence of different maternal environments on germination and emergence.

[‡] WAP, weeks after planting.

[§] Data on height at R6, late-season canopy area, and shattering were not collected in 2012.

shown that higher seed mass (>20 g 100 $seed^{-1}$) can be detrimental to soybean establishment. Reduced soybean population density was observed among the largest seeded lines (22–25 g 100 $seed^{-1}$; Place et al., 2011b). Field emergence plummeted for the largest seed class of certain lines (>22 g 100 $seed^{-1}$), which was attributed to hypocotyl inhibition due to increased soil resistance experienced by large seed (Burris et al., 1973). Since vegetable soybean seed mass of many cultivars exceeds 20 g 100 $seed^{-1}$, and because emergence was particularly low in the present experiments, study of the potential detrimental effect of large seed size on emergence is needed.

Any seed size-mediated effects on early plant growth dissipated mid-season, as evidenced by similar ($P \geq 0.151$) plant height and canopy area between soybean types at 6 WAP (Table 2). Perhaps once plants become autotrophic, differences in early plant growth no longer affect later plant growth. Alternatively, mid-season growth differences are subtle and difficult to detect. These results are consistent with Burris et al. (1973) and Place et al. (2011b), who observed morphological differences in soybean seedlings from different seed sizes were no longer apparent beyond 5 wk after emergence.

Compared to the grain-type entries, vegetable soybean developed more quickly. For example, on average, 6.2 to 7.0 fewer days were needed for vegetable soybean to achieve R1 (Table 2). Days to reach R6, time of commercial vegetable soybean harvest, was 9.5 to 17.9 d fewer than required of grain-type entries. Therefore, the growing season of vegetable soybean is shorter than grain-type soybean, not only because the crop is harvested at an earlier growth stage (i.e., R6 vs. R8), but also because the crop reaches the R6 growth stage earlier than grain-type soybean. This may have agronomic implications to post-harvest field management, late-season weed control, and use of succeeding cover crops or double crops.

In contrast to early-season responses, vegetable soybean produced smaller plants than grain-type soybean at R6. For example, mean plant height and canopy area at R6 averaged 30 and 40% less, respectively, in vegetable soybean compared to grain-type soybean (Table 2). A small plant canopy offers advantages to mechanical harvest of vegetable soybean. Modern fresh legume harvesters can be used successfully in vegetable soybean (Mbuvi and Litchfield, 1994). Podded vegetable soybean can be harvested with a snap bean harvester, whereas shelled vegetable soybean can be harvested with a green pea harvester. Regardless of product type (podded or shelled), harvester efficiency and product recovery declined sharply as plant canopy volume and density increased beyond typical bean and pea plant sizes (Mbuvi and Litchfield, 1994; Mebrahtu and Mullins, 2007). With the exception of plant height, the extent to which additional plant canopy traits (e.g., branching, leaf area index, and pod height) affect mechanical harvest of vegetable soybean have not been reported.

Vegetable soybean entries were far more susceptible to shattering at maturity than grain-type soybean (Table 2). Vegetable soybean entries, particularly imported lines, are older and less agronomically improved than U.S. developed grain-type entries. Although shattering is not necessarily a direct problem for vegetable growers and processors, shattering could be problematic nonetheless since it adds production costs to seed companies; costs which may be transferred to the vegetable industry and consumer.

Table 3. Criteria used to identify candidate lines of vegetable soybean, threshold value of each criteria, and basis for threshold value.

Criteria	Threshold	Basis
Emergence	>36%	above-average field emergence
Height at R6	<66 cm	suitable for machine harvest (Mebrahtu and Mullins, 2007)
Seed mass	>20 g 100 seed^{-1}	seed size threshold for U.S. market (Rao et al., 2002)
Sensory evaluation	acceptable	R6 pods and seeds acceptable to a vegetable processor; specifically, two- to three-seed pods, green pods and seeds, seed free of blemishes, a smooth seed texture, and seed with a sweet and/or nutty flavor

Candidate Lines

Criteria used to identify candidate lines, while not exhaustive, represent critical aspects of vegetable soybean production (Table 3). The process serves as a useful, quantitative step to narrow the list of available germplasm to some of the most promising entries. Of the 136 vegetable soybean entries evaluated in these trials, 72 entries exhibited above-average field emergence, 59 entries had a plant height within the range suitable for mechanical harvest, and 105 entries produced seed that met or exceeded the size threshold for the U.S. market. A total of 17 entries met all three criteria for emergence, height, and seed mass. Of the 17 entries, four entries failed the sensory evaluation because they lacked a sweet and/or nutty flavor. The final 12 entries passing all criteria were from eight different seed sources; six sources being seed or vegetable processing companies and two sources from the public domain (Table 4). Average time from planting to R6 ranged from 70 to 86 d.

Both yield and recovery, defined as the percentage of marketable product relative to total biomass harvested, are additional factors important to the vegetable processing industry. A limited seed supply for these trials made it impossible for an experimental design to accurately measure yield, or recovery, of individual entries. However, in separate research, three vegetable soybean cultivars yielded 8 to 9 Mt ha^{-1} in similar growing conditions (author, unpublished data, 2013). Although beyond the scope of the present work, data on yield of entries in response to different management and environments could serve as additional selection criteria.

Interestingly, entries developed locally (e.g., Illinois and Iowa) were not on the final list of candidate lines. Entries from Iowa State University and University of Illinois met nearly all of the criteria, with the exception of plant height. Take for example the 14 Gardensoy lines released by Dr. Dick Bernard at the University of Illinois. Although the Gardensoy lines are developed specifically for Illinois, average height at R6 of the lines exceeded the machine harvest height threshold by 20 cm (data not shown). As the name suggests, Dr. Bernard developed the vegetable soybean lines for use by home gardeners, and expressed some concern on their suitability for commercial processing (personal communication, 2011).

Table 4. Candidate lines of vegetable soybean identified from analysis of traits observed in 136 entries from field trials conducted in 2011, 2012, and 2013 near Urbana, IL. Source of seed and average days from planting to R6 are included.

Entry	Source	Days to R6
Bukers Favorite	Rupp Seeds, Wauseon, OH	77
Early Hakucho	USDA Soybean Germplasm Collection, Urbana, IL	83
Fledderjohn	Baker Creek Heirloom, Mansfield, MO	72
JYC-2	JYC International, Houston, TX	86
Kegon	USDA Soybean Germplasm Collection, Urbana, IL	77
Midori Giant	Wannamaker Seeds, Saluda, NC	81
Okuhara 1-B	USDA Soybean Germplasm Collection, Urbana, IL	71
Ou yuan tsao shen	USDA Soybean Germplasm Collection, Urbana, IL	70
VS1	anonymous	82
VS7	anonymous	82
Tankuro	Kitazawa Seed, Oakland, CA	77
WSU 729	Washington State Univ., Pullman, WA	82

CONCLUSIONS

By comparing vegetable soybean responses to grain-type soybean, this work puts into perspective the agronomic performance of vegetable soybean germplasm available to growers in the United States. Three years of field experiments showed that germination and emergence of vegetable soybean were poorer than grain-type entries, while seedling growth traits were higher in vegetable soybean. Vegetable soybean developed more quickly than grain-type soybean. In contrast to early growth differences, vegetable soybean produced shorter, smaller plants at the R6 growth stage, a beneficial trait since previous work has shown the importance of relatively small plants for mechanical harvest of vegetable soybean. Seed mass likely accounted for some of the variation in seedling traits. The work also points to areas of future research. Specifically, strategies to improve vegetable soybean emergence are needed. This will involve a greater understanding of the effect of large seed size on crop emergence. Seed size also appears to affect seedling growth, and a greater understanding of how seed size and seedling growth can be leveraged to improve weed suppression would be advantageous, given the limited number of herbicides registered for use in vegetable soybean. Consumer demand for vegetable soybean has been rising globally and the United States has a well-developed soybean industry. Although the United States imports vegetable soybean, American consumers are interested in domestically grown product. The level of additional research and development applied towards vegetable soybean production may be a driving factor of the extent to which the crop is ultimately grown in the United States.

Supplemental Information Available

Supplemental information is available with the online version of this manuscript.

Acknowledgments

Jim Moody managed the experiments and many students provided assistance. I wish to thank Randy Nelson for valuable discussion. This research would not have been possible without support from the vegetable seed and processing industries. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Dep. of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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