Establishment of Selection Criteria for Breeding Sprout Soybeans

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Introduction

Soybeans are a major source of protein and fatty acids in human and animal nutrition. Changes in consumer acceptance, growing global demand and improved processing technologies have promoted the development of the specialty soybean industry. **Soybean sprouts** are an important vegetable consumed in many Asian countries and are gaining popularity in the U.S. To date, breeders have used natto soybeans cultivars to supply sprouts because they share some characteristics such as uniform seed size of < 9 g per 100 seeds. However, sprouts produced by natto soybean could be rejected by sprout manufactures due to different characteristics required by sprout soybeans. There is limited information of sprout soybean characters for breeding purposes. Seed quality and sprout quality studies are needed to facilitate breeding programs in the United States.

Objective

To identify soybean seed and sprout traits that are related to good quality sprouts in order to establish breeding criteria of soybean sprouts.

Materials

Nine small-seeded genotypes were grown at Mount Holly (MH) and Warsaw (W) in 2013 and 1014 (Table 1).

Table 1: Soybean lines for the sprouting study tested in MH and W.

Name	Pedigree	Name	Pedigree
MFS-561	Sprout Cultivar	Glenn	Conventional Cultivar
V05-5973W	NATTO CHECK	V12-1827	V03-0986 x V01-2245
V12-1939	V03-0986 x R04-198	V12-1818	V03-0986 x V01-2245
V12-2249	V01-4937 x R04-198	V12-1764	V03-0986 x V01-2245
V12-1789	V03-0986 x V01-2245		

Methods

- **Planting Methods**

Genotypes were planted in 4-row plots spaced 0.82m with three replications at Warsaw and Mount Holly in Virginia in 2013 and 2014. They all have white flowers to produce white hypocotyl to meet sprout requirement.

- **Sprout trait evaluation**

A total of 350 seeds of each genotype were put into sprouter for 5 days at room temperature (Fig. 1). Water of the sprouter was changed twice per day.

Figure 1: Sprouter (SC-9000TS, Korea)

Figure 2a: High quality sprouts

Figure 2b: Mid quality sprouts

Figure 2c: Low quality sprouts

- **Sprout traits**

High-quality sprout percentage (HQS%), mid-quality sprout percentage (MQS%), low-quality sprout percentage (LQS%) (Fig. 2), high-quality sprout fresh weight, mid-quality sprout fresh weight, hypocotyl length, hypocotyl thickness and sprout yield calculated as grams of fresh high- and mid- quality sprouts produced by a gram of seeds (g/g seed).

- **Seed traits**

Water absorption: 20 g of seeds soaked in 100 ml water for 16 hours. Water absorption = (weight after total water absorption/initial weight) × 100.

Mold test: Ten seeds (washed in sterile water) placed in holes punched on 4% PDA (potato dextrose agar) plates. Two replications. After five days fungi were identified. Mold incidence= (#infected seeds/10) x 100.

Statistical Analysis

Genotype, environment and genotype x environment effects were evaluated using analysis of variance (ANOVA). Location and year factors were combined into one environment term for a total of 3 environments. Tukey's HSD test was used to show which variable differed significantly at P=0.05. Correlation coefficient among variables and principal component analysis were calculated using JMP statistical version 11.0 by the SAS institute Inc.

Results

- **Seed trait results**

Water absorption: All genotypes had a water absorption between 223 and 231% and it was not significantly different neither among genotypes nor among environments.

Mold test: It showed that six fungus genera widely distributed among genotypes: *Penicillium* sp., *Epicoccum* sp., *Fusarium* sp., *Alternaria* sp., *Aspergillus* sp. and *Mucor* sp. There were not significant differences in seed-born fungus incidence among genotypes or environments. Fungus incidence average was 52.78% in 2013 and 63.12% in 2014.

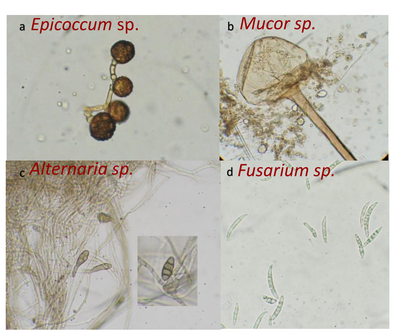

Figure 3: Asexual structures of a) *Epicoccum* sp. b) *Mucor* sp. c) *Alternaria* sp. and d) *Fusarium* sp.

- **Sprout trait results**

Sprout traits were significant different among genotypes. Most of the genotypes were significant thicker than MFS-561 and had higher HQ and MQ sprout fresh weigh than MFS-561. **V12-1789, V12-2249** and **V12-1939** could be considered as sprout variety candidates because they were 9-10 g/100 seeds, and showed better sprout characteristics than MFS-561. Cracked cotyledons and abnormal seedlings are the two main constraints in soybean sprout quality which generated a high MQS percentage and high MQS fresh weight.

Table 2: Sprout traits of genotypes with better quality sprout in W 2013, W 2014 and MH 2014.

Name	†Seed Size g/100 seeds	HQS (%)	†MQS (%)	†LQS (%)	†Fresh weigh HQS (g)	†Fresh weigh MQS(g)	Hypocotyl Length (cm)	Yield (g/g seeds)	Hypocotyl Thickness (mm)
V12-1818	11.2c	58.8a	26.3c	14.9b	172.3 a	64.5d	13.9ab	5.9ab	1.9ab
V12-1789	10.9cd	57.5ab	31.4bc	11.1b	154.1ab	71.3cd	14.4ab	5.9abc	1.7abc
V12-1827	11.9b	57.5ab	30bc	12.5b	168.8a	75.7bcd	15.7a	5.8abcd	1.9a
V12-2249	10.5d	51.8abc	37.1ab	11.1b	128.2bcd	86.5abc	13.9ab	5.9abc	1.7abc
Glenn	14.2a	49.0abc	35.1abc	15.9b	146.9abc	98.8a	12.8b	4.9d	1.8ab
V12-1939	9.6e	47.3abc	42.5a	10.2b	118.3cde	97.8a	14.2ab	6.4a	1.7ab
MFS-561	8.3f	45.0bcd	37.4ab	17.6b	91.2ef	65.1d	12.9b	5.3bcd	1.5c
V12-1725	10.5d	42.2cd	38.9ab	18.8b	116.4def	93.6ab	13.8ab	5.7abcd	1.8ab
V12-1764	9.1e	34.1d	33.3bc	32.6a	87.6f	76.1bcd	12.7b	5.1cd	1.7abc
Average	10.7	49.2	34.7	16.1	131.5	81.0	13.2	5.7	1.7

*Different letters in the same column indicate significant difference at the 0.05 probability level †affected by Genotype x Environment interaction.

- **Environmental effect**

Characters related to sprout growth were affected by environments. One year storage may affect germination rate, hypocotyl length, sprout yield and HQS percentage. Hypocotyl length, HQS % and sprout yield were not affected by genotype x environment interaction.

Table 3: Sprout Traits of seeds in W 2013, W 2014 and MH 2014.

Environment	†Seed size (g)	HQS%	†MQS%	†LQS%	†Fresh weigh HQS(g)	†Fresh Weigh MQS (g)	Length (cm)	Thickness (mm)	Sprouts yield (g/g seed)
W-2013	9.9c	48.8a	28.6c	22.6a	111.2c	56.9 c	11.3b	1.8	4.9 b
MH-2014	11.2a	45.6ab	41.1a	13.3b	131.2b	101.38 a	15.1a	1.7	5.9 a
W-2014	11.0b	53.3b	34.3b	12.4b	152.2a	84.81 c	14.8a	1.8	6.2 a

*Different letters in the same column indicate significant difference at the 0.05 probability level †affected by Genotype x Environment interaction.

- **Variables Correlation**

Higher hypocotyl length, high-quality and mid-quality sprout percentage would determine higher sprout yield. Seed size, hypocotyl thickness and water absorption were not significantly correlated to any variables, indicating that they were relatively independent variables.

Table 4: Correlation coefficient among sprout traits in W 2013, W 2014 and MH 2014.

	Seed Size (g)	HQS%	MQS %	LQS%	Fresh weigh HQS(g)	Hypocotyl Length	Hypocotyl Thickness	Water absorption%	Sprout yield g/g seed	Mold test%	Fresh Weigh MQS (g)
Seed Size (g)	1										
HQS %	0.30	1.00									
MQS%	-0.05	-0.58	1.00								
LQS%	-0.32	-0.67*	-0.22	1.00							
Fresh weigh HQS(g)	0.57	0.87*	-0.44	-0.64*	1.00						
Hypocotyl Length	0.17	0.08	0.41	-0.47	0.33	1.00					
Hypocotyl Thickness	0.37	0.16	-0.15	-0.05	0.33	0.00	1.00				
Water absorption%	-0.20	-0.29	0.04	0.32	-0.35	-0.35	-0.09	1.00			
Sprout yield g/g seed	-0.02	0.52	0.12	-0.73*	0.59	0.60*	0.03	-0.28	1.00		
Mold test%	0.02	-0.03	0.10	-0.06	0.02	0.28	-0.02	0.01	0.03	1.00	
Fresh Weigh MQS (g)	0.39	-0.33	0.78*	-0.32	-0.05	0.51	0.02	-0.08	0.31	0.05	1

*significant correlation between variables.

- **Principal Component Analysis**

Four eigen values (greater than 1) explained a total of 79% of the variance in the 11 original variables. Where, *high- and mid- quality sprout percentage, high- and mid quality sprout fresh weight, seed size and thickness* could be considered the most important variables for evaluation of soybean sprout quality in breeding programs.

Conclusions

The soybean sprout breeding criteria are high-quality sprout >45%, mid-quality sprout < 34.7%, high-quality sprout fresh weight > 91.2 g, mid-quality sprout fresh weight> 65.1 g, sprout yield of 5.3 g/ g seed, hypocotyl length >13 cm, hypocotyl thickness> 1.5 mm and molds < 60%.