AAFC RESEARCH BRANCH
Research Project Final Report
Developing Innovative Agri-Products Program (Vote 10 Funding)

Project Title:	**Activity A.1:** **Obtain new germplasm material that can be used to address the needs for improved nutraceutical and/or agronomic value.**
Start Date (yyyy-mm-dd):	2010-04-01
Expected End Date (yyyy-mm-dd):	2013-03-31
Actual End Date (yyyy-mm-dd):	2013-03-31
Principal Investigator (PI):	Sevita International – David Hendrick/Jim McCullagh

Short Executive Summary of report:

Sub-activity 1.1 – Natto soybean seed size and seed yield limitation
Soyfood cultivars are selected by Asian food manufacturers for their end-use function in the production of tofu, soymilk or natto. Canadian exporters and growers are also interested in profitable production, especially improved seed yield.
Natto is a fermented soybean product for producers specifically require small seeded soybean. Natto-type soybeans are highly specialized and serve niche markets. As a result, little public research and development targets natto-type soybean. Since smaller seeded soybean cultivars are lower yielding, this sub-activity investigated breeding approaches and physiological approaches to increase yield. A small x large seed cross was compared to a small x small seed cross. It was found that the small x large seed cross produced a wide range of seed sizes with somewhat higher yield. Five natto lines have been retained for further testing as potential cultivars. From physiological studies, it was found that seed yield was optimum with a seed weight of 15 grams/100 seeds. Using shading, it was found that natto soybean plants are not source (sunlight absorption) limited, that is, they did not respond differently to shades reducing sunlight compared to large seeded cultivars.

Sub-activity 1.2 – Enhancing the food grade quality of short-season soybeans and Ontario, PEI and Austria
The objective of this project was to develop new germplasm from crosses between Canadian cultivars and soybean germplasm lines and cultivars from Canada and overseas with beneficial traits for the food grade and nutraceutical markets. Every year, a number of biparental crosses were made in the growth room at the University of Guelph to produce F1 seeds that were used for the production of the breeding populations. Traits of interest under this sub-activity included high yield, high protein content, absence of lipoxygenase and modified fatty acid profiles. Single plants were selected in F4, single rows in F5 and F6 lines were selected based on preliminary yield trials in one location. The F7 and F8 lines were selected based on advanced and private yield trials. As a result of this project, a number of soybean cultivars without lipoxygenase and low linolenic acid content have been developed. Such traits improve the taste of tofu and soymilk by reducing or removing the "beany" taste that comes as result of polyunsaturated fatty acid oxidation in the soybean food products. In addition, cultivars with enhanced vitamin E content adapted to Ontario conditions as well as number of high yielding food grade cultivars have been developed and released to industry.
Tofu and natto trials were conducted on Prince Edward Island - as a new region for production of food soybean cultivars - to evaluate their performance in this environment. Several genotypes, specifically DH863 and DH710, performed well in the tofu and natto trials, respectively.

Sub-activity 1.3 – Selection of high tocopherol soybean lines
For objective 1, a multi-location replicated trial was initiated in 2011 at three sites in Eastern Canada with twenty 2800-2900 CHU cultivars or advanced selections from Sevita International. Data were collected during the 2011 and 2012 seasons and all samples were analyzed for tocopherol concentrations. At the end of the trial, all data from the six environments were analyzed using various statistical tools, including GGE genotype-by-trait biplots and mean tocopherol concentration versus coefficient of variation biplots to assess tocopherol variation and

stability among genotypes evaluated. The data demonstrated that among the advanced material from Sevita International there is a large variation in α-tocopherol concentration, values ranging between 7.9 and 38.2 µg/g, representing a 382% difference in concentrations across sites and across genotypes. The variation observed for other tocopherols was smaller, being of 95, 48 and 47%, for delta-, gamma- and total tocopherol. Despite the presence of significant genotype by environment interactions for all tocopherols, the ranking of genotypes was often quite stable across environments. Genotypes with high and stable tocopherol concentrations were identified, which were HS0511H32, DH530, DH715L and DH410SCN for α-, δ-, γ- and total tocopherol.
For objective 2, correlations between 30 important agronomic, food and nutraceutical properties were calculated for 156 food grade soybean samples collected from multi-location trials in 2010, 2011 and 2012. These variables included: tocopherols, isoflavones, lutein, soyasapogenols, oil, protein, sucrose, stachyose and raffinose, fatty acid profile, seed yield, seed weight, % emergence, days to flowering, days to maturity, plant height, lodging and white mould incidence. All compositional data were analyzed using wet chemistry. There were a total of six positive and seven negative correlations for α-tocopherol, seven positive and six negative for δ-tocopherol, seven positive and ten negative for γ-tocopherol and six positive and eight negative for total tocopherol. The correlations with the highest Pearson coefficients for α-tocopherol were those with days to maturity and days to flowering (i.e. r=-0.54 and -0.50, respectively). Similar strong correlations with days to maturity were also observed for γ-tocopherol and total tocopherol (i.e. r=-0.72 and -0.52, respectively). A negative correlation was also observed between these three tocopherols and seed yield (i.e. r ranging between -0.40 and -0.64). Although some significant correlations were observed between tocopherols and some other nutraceutical traits such as isoflavones and lutein, none had a Pearson correlation coefficient greater than ±0.40. No correlation between tocopherols and other important seed traits such as protein and oil content was observed.

Sub-activity 1.4 – Development of markers for cytokinin based yields in soybeans
Seed composition can affect the function and flavour of soymilk and tofu. Breeding populations varying for protein profile, sucrose content and lipoxygenase enzymes were developed and breeding lines evaluated. One line has been retained with higher levels of sucrose, SE06-0337ML-1. The line SE06-0337ML-3 has higher hydrolysable carbohydrates, a trait useful for miso production. Lipoxygenase null lines produce soymilk with a less “beany” flavour and one lipoxygenase free line has been retained, SQ05-0026M-3lx-3.
Eight lines will be further evaluated for their potential as new cultivars.

A. Research Progress and Accomplishments (to date in relation to expected milestones and deliverables / outputs)

- Include brief summary of:
  - Introduction, literature review, objectives, milestones and deliverables / outputs.
  - Approach / methodology (summary by objectives).
- Include results and discussion (overview by objectives and milestones), next steps and references.

Sub-activity 1.1 - Natto soybean seed size and seed yield limitation
Small seeds are desirable for natto, a traditional Japanese food made from cooked fermented whole soybean seeds. Exporters from Eastern Canada typically use 5.5 millimeter (mm) (#14) round-hole screens to condition natto soybean. Small-seeded natto cultivars yield less than oilseed cultivars in Eastern Canada. From 17 trials grown in Ontario and Quebec in 2000 to 2002, natto cultivars yielded 68 to 87% of a check oilseed cultivar. It would be useful to use high-yielding, larger-seeded oilseed cultivars as sources of high yield in crosses with natto cultivars. Recently, some natto exporters have expressed an interest in somewhat larger-seed lines, especially if the seed yield could be increased as a result. In the past, studies have been undertaken to look at relaxing seed size requirements during mass selection of populations resulting from single and three way crosses. Selection for seed size did not significantly affect seed yield or days to maturity, but did result in significantly reduced seed weight. The seed yield of natto selections remained at about 80% of the oilseed check cultivars used in this study.

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Objective

The objective of this sub-activity is to develop new soybean germplasm that could lead to increased yield in specialty "natto" type soybean by investigating the relationship between seed size and yield in two populations (deriving from small x large seed size and small x small seed size crosses) and to understand the factors limiting yield in natto soybean lines. This will be addressed in natto breeding programs or through agronomic manipulation. Knowledge will result in improved objectives for natto breeding and improved production practices. Higher yielding natto lines could increase overall production in Eastern Canada and increased exports of the specialty identity preserved crop to international markets.

Performance Indicator

Natto cultivars were identified in the course of evaluating the two populations, which will be commercialized by Sevita International.

Methodology – Breeding study

Recombinant inbred lines (RILs) from the two populations were grown in Ottawa, ON, on the Experimental Farm as well as at Sevita International's Inkerman nursery site, located outside of Winchester, ON. Data was collected on seed size and agronomic characteristics and a combined analysis was carried out, all measurements were adjusted to 13% moisture.

Results and Discussion – Breeding Study

The two populations differed greatly for their seed size distribution. The lines retained for testing in 2012 were selected for small seed size and seed yield. The population deriving from the small x small cross (X5076) has smaller seeds as expected while the population deriving from the small x large cross (X5077) has a broader seed size distribution. Seed yield was lower for the X5076 population over the three year analysis. From this study, the best approach to combine yield and small seed size is to use high yielding medium seed sized parents and select for smaller seed sized progeny rather than the larger seeded parent that was used in X5077, since this would result in more progeny in the acceptable natto size range.

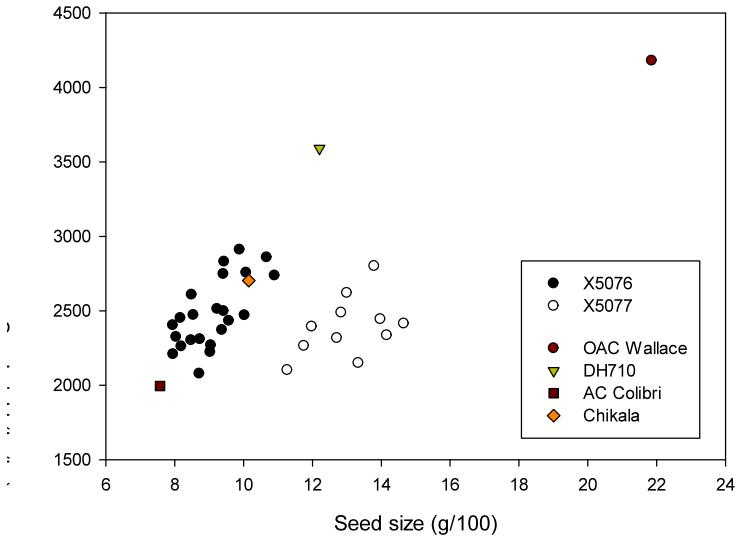

Figure 1: Seed yield by seed size of parental varieties, OAC Wallace, DH710, AC Colibri, and Chikala compared to the progeny of cross X5076 and X5077.

Methodology - Physiology Experiments to determine the influence of natto soybean seed size on growth characteristics

Selection of lines

Eight lines were selected from cross X5077 which was between the natto cultivar AC Colibri and the oilseed cultivar AC Orford (Table 1). The lines were selected primarily for seed size measured as the weight (g) per 100 seeds and total seed yield with secondary characteristics being plant height and branch number measured as the % of the total yield contributed by the branches.

Table 1. Characteristics of natto lines selected from cross X5077 for the physiology experiments.

Natto line	Height cm	Branch Yield %	Total Yield kg/ha	Maturity days	Seed Weight g/100	Lodging (1-5)
X5077-1-1-1-18-B	78	75.6	2028	119	10.5	1.8
X5077-1-1-1-93-B	98	29.7	2418	116	10.5	2.4
X5077-1-1-1-94-B	103	41.9	2781	116	9.8	2.2
X5077-1-1-1-57-B	80	27.9	3135	114	7.6	2.2
X5077-1-1-1-65-B	84	42.6	2144	115	13.7	3.1
X5077-1-1-1-194-B	91	12.1	2243	116	12.8	3.1
X5077-1-1-1-68-B	95	56.1	3086	115	14.7	1.8
X5077-1-1-1-150-B	88	13.3	3603	119	14.1	2.3

GA = Growth Analysis study done prior to harvest.
Lodging 1= standing; 5 = flat.

There was one group with small seed size and one with moderate seed size. Within each group the yield varied from high to low.

The eight lines from cross X5077 and their parents were grown in a randomized block design yield trial in 2010 to 2012. During the season, plant growth was monitored by removing a sample and dissecting it to determine leaf area and component dry weights. Seed yield and yield components were determined at harvest. Leaf photosynthesis was determined several times during the growing season to examine the relationship between the plant growth, photosynthetic rate and seed yield. Shade fabric was used to limit the amount of solar radiation reaching the plants to further examine the source/sink relationship in natto. A seed size experiment was done on three commercially available natto lines to determine the influence of planted seed size on yield. The seed was screened to remove the smallest one third of the seed. An experiment was done to determine if spraying a growth regulator on the plants at the beginning of pod development would increase pod retention and yield.

Result and Discussion

Agronomic Trial Results - Seed size and yield (Figure 2 & Figure 3):

In 2010, there were seven lines with greater yield than AC Colibri and two lines with greater yield than AC Orford. The line with the highest yield had a seed weight of 16.4 g per 100 seed while AC Colibri had a seed weight of 8.0 g per 100. In 2011, there were nine lines with a greater yield than AC Colibri and one line with a greater yield than AC Orford. The highest yielding line in 2011 had a seed size of 12.8 grams per 100 seed. The growing season in 2012 was extremely dry and mean seed yield was reduced by 37% in comparison to the mean seed yield of 2010 and 2011. In 2012, all lines had greater yield when compared to AC Colibri and seven lines had greater yield when compared to AC Orford. The highest yielding line in 2012 had a seed size of 14.3 g per 100 seed.

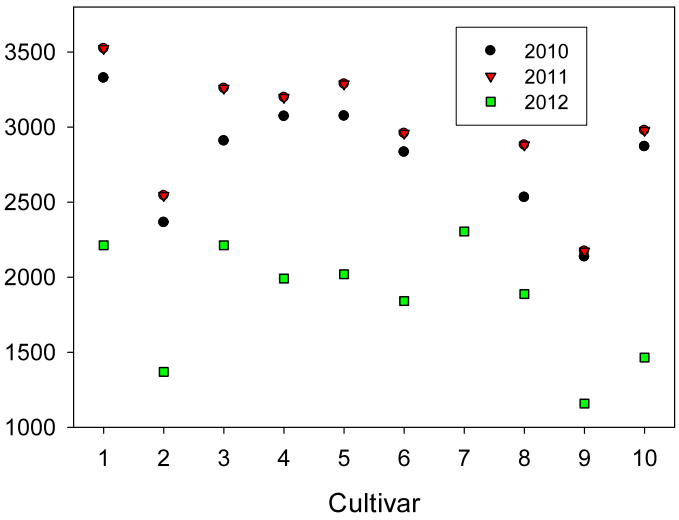

Figure 2: Yield differences across years

Line/cultivar:

1 = X5077-1-1-1-18-B *2 = X5077-1-1-1-57-B*
3 = X5077-1-1-1-65-B *4 = X5077-1-1-1-68-B*
5 = X5077-1-1-1-93-B *6 = X5077-1-1-1-94-B*
7 = X5077-1-1-1-150-B *8 = X5077-1-1-1-194-B*
9 = AC Colibri *10=AC Orford*

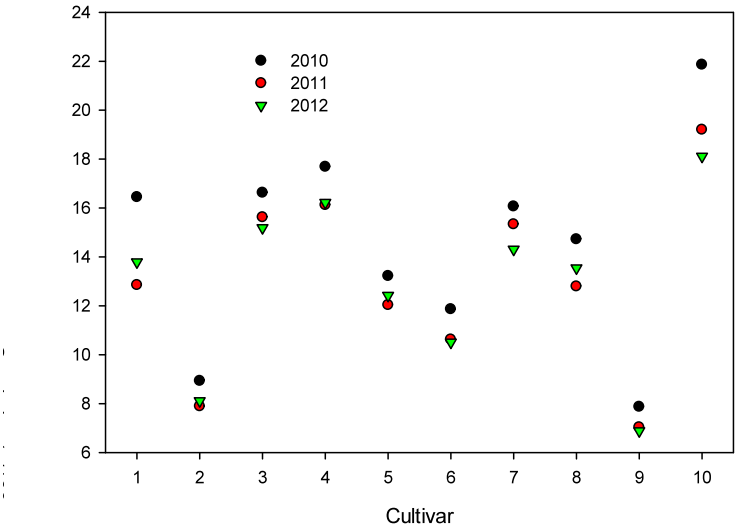

Figure 3: Seed size differences across years

Line/cultivar:

1 = X5077-1-1-1-18-B *2 = X5077-1-1-1-57-B*
3 = X5077-1-1-1-65-B *4 = X5077-1-1-1-68-B*
5 = X5077-1-1-1-93-B *6 = X5077-1-1-1-94-B*
7 = X5077-1-1-1-150-B *8 = X5077-1-1-1-194-B*
9 = AC Colibri *10=AC Orford*

There was a quadratic relationship between seed size and yield within the X5077population with yield increasing as seed size increased up to 16.5, 14.0, and 14.0 g/100 seed in 2010, 2011 and 2012, respectively (Figure 4).

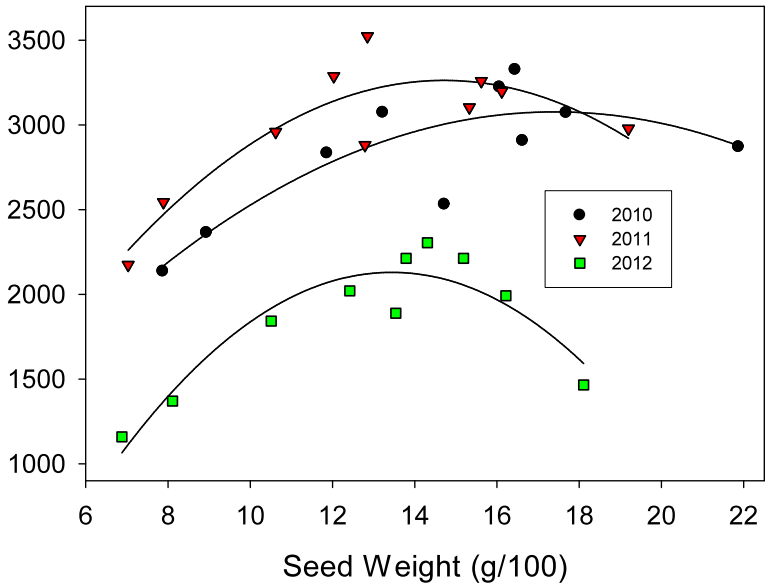

Figure 4: Relationship between seed weight at planting and seed yield at harvest

Agronomic Trial Results - Plant Growth

Seed size at planting affected early plant growth up to the beginning of flowering. Plants from large seed had greater leaf area, more weight per plant, and were taller (Fig 4). This resulted in more total biomass per square meter, which translated into higher seed yield at harvest.

Plants with the largest leaf and total weight at flowering resulted in the highest yield (Fig 5.). This is a cubic relationship meaning that there is an optimum leaf weight or plant size to achieve the highest yield. These relationships held up for three years of the test.

The lines with the greatest leaf area during early vegetative growth had the highest final seed yield. A method to increase seed yield in natto would be to select for large plant size within a cross at flowering and combine this with selecting for small seed size at harvest.

Another possible way to increase yield in natto would be to eliminate the smallest seed from the seed lot. This may result in larger plants at flowering and greater seed yield at harvest.

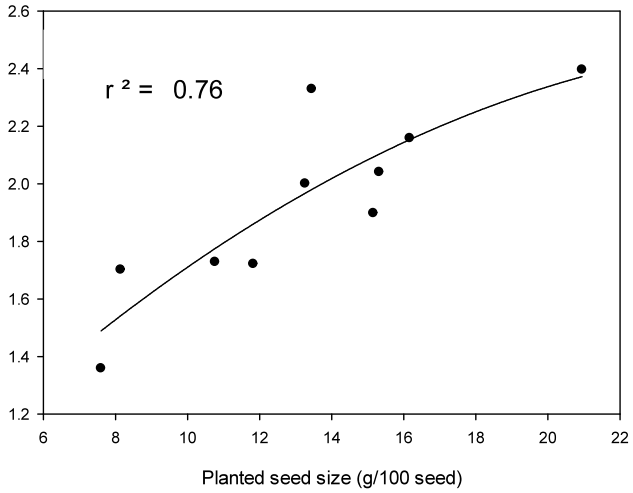

Figure 5: The influence of planted seed size on leaf weight at flowering per plant

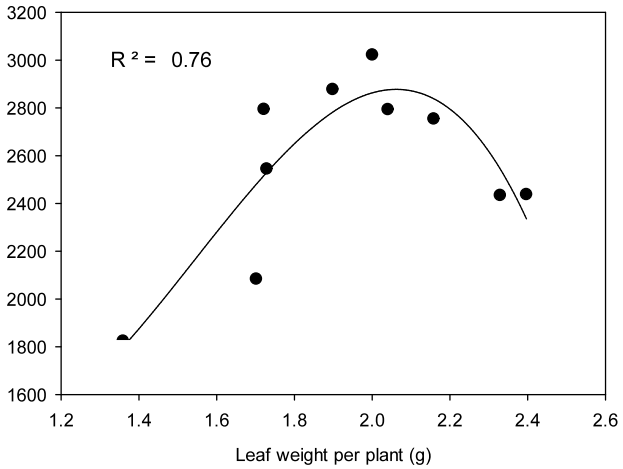

Figure 6: The influence of leaf weight at flowering per plant on seed yield at harvest.

Agronomic Trial Results - Photosynthesis:
There were significant differences among lines for photosynthetic rate per leaf area but there were no consistent trends across the years. Both 2010 and 2011 had higher average photosynthetic rates than the dry year 2012. There was no clear association between photosynthetic rate and yield. The differences in yield were not influenced by the photosynthetic rate per area in natto.

Agronomic Trial Results - The effect of shade on plant growth and yield:
Shades were used to reduce the amount of net radiation reaching the plant by 40 and 60%. There were significant differences among the cultivars for all of the parameters examined (Table 2) and among the parameters affected by the different light levels reaching the plants. There were no significant interactions between the light level and the parameters measured, indicating that that all plants responded in a similar fashion to reduced light intensity. When the net radiation reaching the plants was reduced by 40 and 60 % with shade fabric, seed yield decreased by 22 and 45%. This indicates that natto soybean is not source limited. To increase natto yield cultivars must be selected with a greater number of seeds per plant but currently the harvest index (the ratio of seed yield to biomass yield) is already greater than 50% indicating that the plant is very efficient at converting biomass into seed yield. Plant size at flowering should be a criterion for selection since it would increase total biomass and may increase yield.

When the amount of radiation reaching the soybean plants was reduced, all cultivars responded the same way in both years for many characteristics. The exception to this was seed yield and harvest index where four cultivars changed rank substantially between years for yield resulting in changes in harvest index. Seed size did not change substantially among years except in 2012 when the drought reduced overall seed size. As the amount of radiation reaching the plant decreased, all plants produced more of the yield on the main stem and yield decreased by an average of 21 and 40% for radiation reductions of 40 and 60%, respectively.

Table 2. Analysis of Variance table for the influence of shading on plant growth characteristics.

	Nodes/plant	Branches /plant	Main Stem Yield	Harvest Index	Seed Yield	Seed Weight
2011						
Line	**	**	**	**	**	*
Shade	*	**	**	*	**	NS
Line x Shade	NS	NS	NS	NS	NS	NS
2012						
Line	*	NS	**	**	**	**
Shade	NS	**	**	NS	**	NS
Line x Shade	NS	NS	NS	NS	NS	NS

*,** = significant at the 0.5 and 0.01 levels of probability, NS = not significant.

Screening for Seed Size:
Three natto cultivar seed lots were screened to remove the smallest one third of the seed. This test was done in 2012 at Ottawa (O) and Inkerman (I) so the results may reflect the drought conditions in the area. Screening for seed size varied with cultivar and location (Figure 7). On average, reducing the number of small seeds from the seed lot did not make a significant difference in seed yield. The exception was HS06-03 that yielded 500 kg/ha more at Inkerman when the smallest one third of the seed were removed. This experiment should be repeated.

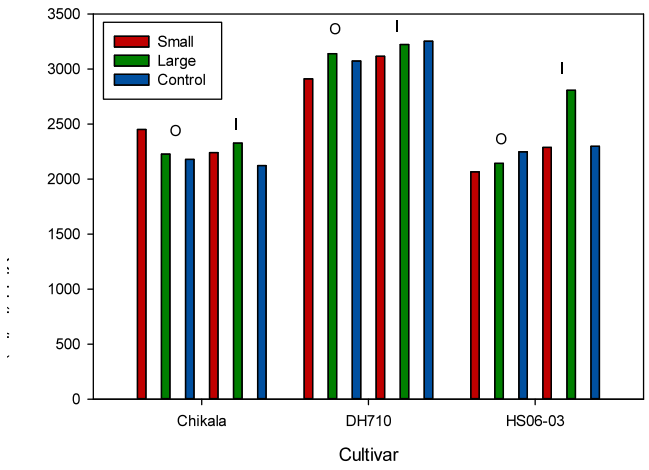

Figure 7: Influence of planted seed size (smallest one third, largest two thirds and control) on seed yield at O=Ottawa, I=Inkerman

Plant growth regulation and yield:
Spraying AC Colibri plants with a growth regulator at the beginning of seed development R3 to R4 did not affect the harvested seed yield of the plots neither in 2011 nor 2012. The application of cytokinin or boron did increase the number of pods retained on the plants in 2012, but this did not translate into greater yield.

Conclusions and Next Steps

- There are lines from the X5077 population with greater yield and smaller seed than AC Orford, but there are no lines with similar seed size and yield as AC Colibri. These lines all had larger seed size than 10 grams per 100 seeds.
- Seed size is associated with yield. As seed size increases, yield increases up to about 150 grams per 1000 seeds in this population.
- Planted seed size affects spring growth rate and early plant size and early leaf area. The larger the seed size, the larger the plant. The planted seed size was correlated with the leaf area and the plant dry weight at flowering. The leaf area and the plant biomass at flowering, even in a very dry year, correlated with seed yield at harvest.
- Greater number of pods per plant is associated with smaller seed size. Yield increases with pod number up to a point.
- Natto soybean plants are not source limited as shown by the shade experiments. Within this population, yield was not associated with the photosynthetic rate. All lines responded the same way when the radiation reaching the plant was reduced by 30 or 60% with the use of screens. A reduction in net radiation did not result in the same level of reduction in yield. When the net radiation reaching the plants was reduced by 40 and 60 % with shade fabric, seed yield decreased by 22 and 45 %. This result and the results from the measured photosynthesis indicate that natto soybean reaches a maximum photosynthetic efficiency at relatively low light (1400 to 1600 micro Einsteins).
- The results of screening seed to remove the smallest third of the seed varied with cultivar and location. One line, HS06-03, produced 500 kg/ha more seed yield when screened at the Sevita International Inkerman nursery location. This experiment should be repeated.
- Spraying the plants with the growth regulator cytokinin did not increase harvested yield in natto.
- Identification of five Natto lines for commercialization resulted in the course of the two population

evaluations (Figure 8 & Table 3):

1. X5076 -1-1-1-139,
2. X5076-1-1-1-185,
3. X5076-1-1-1-240,
4. X5076-1-1-1-245,
5. X5076-1-1-1-262.

These cultivars will now undergo further grower trials, food and end-user testing to determine if they will be commercialized by Sevita International.

Table 3. Summary of AAFC natto trail results from 2012. Three locations; Inkerman, Ottawa and Elora.

Entry	Name	Yield (bussel/ acre)	Yield (kilogram/ hectare)	Rank	Predicted Yield (%)	Days to Maturity	Plant Height (cm)	Lodge Score (1-5)	100 Seed Weight(g)	Oil (%)	Protein (%)	Sucrose (%)	Leaf Shape	Colour Flower	Colour Pubescence	Colour Hilum	Trait Objective
	X5076-1-1-1-245-B	31.2	2078	37	110.0	105	62	1.6	8.7	20.5	40.9	5.3	L	P	G	Y	Natto Seed size
	AC Colibri	29.9	1995	38	105.6	105	59	1.9	7.6	19.8	37.0	5.8	L	P	G	Y	Early maturity Natto
	X5076-1-1-1-139-B	34.6	2309	28	107.4	108	63	1.8	8.7	19.8	41.3	5.7	O-L	P	G	Y	Natto Seed size
	X5076-1-1-1-240-B	34.5	2302	29	107.1	108	75	2.4	8.5	20.0	40.9	5.8	L-O	P	G	Y	Natto Seed size
	X5076-1-1-1-195-B	34.0	2268	30	105.5	108	63	1.5	9.1	20.9	40.9	5.5	L-O	P	G	Y	Natto Seed size
	X5077-1-1-1-156-B	31.5	2102	36	97.8	108	59	1.6	11.3	20.1	35.0	5.4	L	P	T	Y	Natto Seed size
	X5076-1-1-1-185-B	36.0	2403	22	107.5	109	69	1.8	7.9	20.9	39.3	5.9	L	P	G	Y	Natto Seed size
	X5077-1-1-1-215-B	35.0	2335	25	104.4	109	59	1.5	14.2	18.7	37.3	5.1	O	P	G	Y	Natto Seed size
	X5077-1-1-1-24-B	33.9	2264	31	101.2	109	66	1.7	11.8	18.6	37.4	5.2	O	P	G	Y	Natto Seed size
	X5076-1-1-1-4-B	33.9	2261	32	101.1	109	65	1.6	8.2	20.4	40.2	5.9	L-O	P	G	Y	Natto Seed size
	X5076-1-1-1-102-B	33.4	2223	33	99.4	109	60	1.5	9.0	21.4	39.9	5.6	O	P	G	Y	Natto Seed size
	X5076-1-1-1-92-B	33.1	2208	34	98.8	109	67	1.7	8.0	20.6	40.8	5.4	L-O	P	G	Y	Natto Seed size
	X5076-1-1-1-90-B	37.7	2512	13	104.2	111	67	1.7	9.2	19.8	41.6	5.5	L-O	P	G	Y	Natto Seed size
	X5076-1-1-1-256-B	36.8	2451	18	101.7	111	71	2.1	8.2	19.6	41.3	6.1	O	P	G	Y	Natto Seed size
	X5076-1-1-1-107-B	36.5	2433	20	101.0	111	69	1.6	9.6	20.8	40.3	5.8	O	P	G	Y	Natto Seed size
	X5077-1-1-1-214-B	32.2	2149	35	89.2	111	59	1.3	13.3	18.0	38.3	5.0	O-L	P	G	Y	Natto Seed size
	Chikala	40.5	2703	10	108.2	112	65	1.5	10.2	19.7	37.5	5.7	L	P	G	Y	Natto
	X5076-1-1-1-133-B	37.5	2498	14	100.1	112	61	1.7	9.4	21.0	40.7	5.4	L	P	G	Y	Natto Seed size
	X5077-1-1-1-144-B	36.6	2444	19	97.9	112	66	1.6	14.0	18.5	37.2	5.4	O	P	G	Y	Natto Seed size
	X5077-1-1-1-116-B	36.2	2414	21	96.7	112	60	1.5	14.7	18.7	37.5	5.3	L	P	G	Y	Natto Seed size
	X5077-1-1-1-55-B	35.9	2394	23	95.9	112	68	1.7	12.0	19.0	36.4	5.1	L-O	P	G	Y	Natto Seed size
	X5076-1-1-1-155-B	35.6	2371	24	94.9	112	60	2.0	9.4	20.7	41.5	5.3	O	P	G	Y	Natto Seed size
	X5076-1-1-1-110-B	34.9	2324	26	93.1	112	65	2.0	8.0	20.0	40.8	5.7	L	P	G	Y	Natto Seed size
	X5076-1-1-1-262-B	41.0	2737	9	105.9	113	73	2.0	10.9	21.1	41.3	5.6	O	P	G	Y	Natto Seed size
	X5077-1-1-1-54-B	37.3	2488	15	96.3	113	58	1.3	12.8	20.1	34.7	5.5	O	P	T	Y	Natto Seed size
	X5076-1-1-1-188-B	37.1	2472	16	95.7	113	73	2.1	8.5	20.9	39.2	5.9	O-L	P	G	Y	Natto Seed size
	X5076-1-1-1-108-B	39.1	2608	12	97.7	114	70	1.6	8.5	21.2	39.9	5.6	O	P	G	Y	Natto Seed size
	X5076-1-1-1-253-B	37.0	2470	17	92.5	114	65	1.8	10.0	19.3	42.6	5.5	L	P	G	Y	Natto Seed size
	X5077-1-1-1-142-B	34.7	2317	27	86.8	114	61	1.5	12.7	19.0	36.4	5.2	L-O	P	G	Y	Natto Seed size
	X5077-1-1-1-74-B	42.0	2800	6	101.6	115	69	2.0	13.8	19.5	35.6	5.4	O-L	P	G	Y	Natto Seed size
	X5076-1-1-1-237-B	41.3	2756	7	100.0	115	72	1.9	10.1	21.1	40.2	3.9	O	P	G	Y	Natto Seed size
	X5076-1-1-1-159-B	42.4	2830	5	99.4	116	71	1.8	9.4	19.9	42.5	5.3	L	P	G	Y	Natto Seed size
	X5076-1-1-1-184-B	41.2	2747	8	96.6	116	71	1.9	9.4	19.6	42.4	5.6	L	P	G	Y	Natto Seed size
	X5077-1-1-1-115-B	39.3	2620	11	92.2	116	71	1.7	13.0	19.1	37.3	5.0	O	P	T-G	Y	Natto Seed size
	X5076-1-1-1-209-B	43.7	2912	3	99.4	117	71	2.4	9.9	20.9	41.0	5.7	O	P	G	Y	Natto Seed size
	X5076-1-1-1-35-B	42.9	2860	4	94.8	118	77	1.5	10.7	20.5	40.5	6.0	O	P	G	Y	Natto Seed size
	DH710	53.9	3591	2	101.5	124	83	2.4	12.2	19.8	38.9	5.4	L	W	G	Y	Natto
	OAC Wallace	62.7	4181	1	115.3	125	73	1.6	21.9	21.3	36.2	6.2	O	P	T	BR	High Yield, Tocopherols
	Average	37.8	2522			112	67	1.8	10.6	20.0	39.3	5.5					
	Check Average	**46.7**	**3118**			**117**	**70**	**1.8**	**12.9**	**20.2**	**37.4**	**5.8**					
	Coefficient of Variation	10.24	10.2			2.8	9.9	20.12	12.25	4.59	4.296	7.71					
	Least Significant Differences	2.83	189.1			2.28	5.6	0.25	0	0	0	0					
	Number of locations	7	7			7	7	7	7	7	7	7					

Yield adjusted to 13% moisture
Lodging at maturity: (1-5) 1=standing, 5=flat
Flower colour: P=purple, W=white, M=mixed
Pubescence colour: T=tawny, G=grey, LT=light tawny, M=mix
Leaf shape: O=oval, L=lanceolate(narrow), OL,LO=mixed

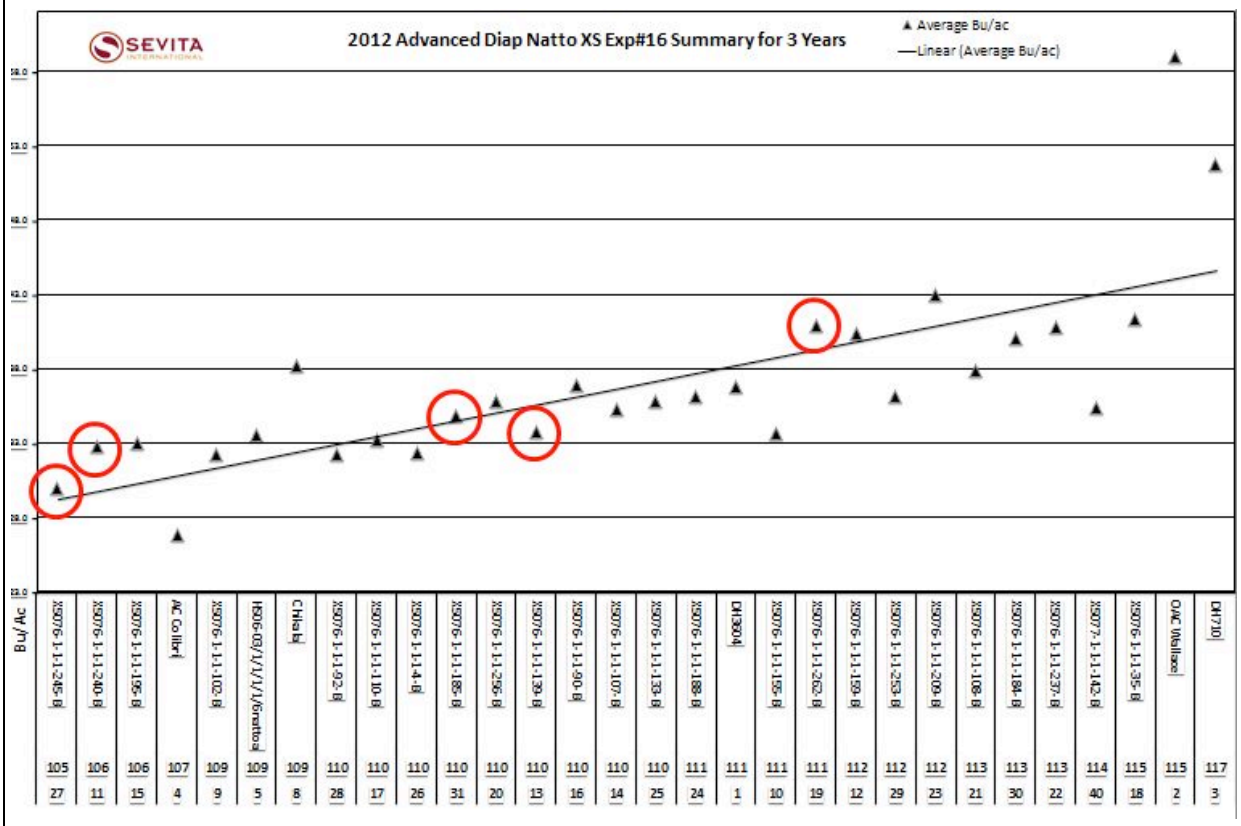

Figure 8: 2010-2012 Yield summary for extra small natto varieties resulting from DIAP Activity A1 Sub-activity 1.1

Sub-activity 1.2 Enhancing the food grade quality of short season soybeans for Ontario, PEI and Austria

Objective

The objective of this sub-activity was to enhance the food grade quality of short season soybeans in Ontario, Prince Edward Island and Austria. Varieties offering enhanced nutrition, health and processing traits without sacrifice to yield or agronomic performance will provide Canadian soybean producers and exporters opportunities to capture increased market share and to open new markets, e.g. emerging nutraceutical markets.

Performance Indicators

To insure commercial viability, food quality testing was undertaken with established laboratories/researchers specifically dedicated to food grade soybean and soyfood evaluation. Tofu cultivars were identified in the course of evaluations to be sent for evaluation by Japanese testing facilities and end-users to determine superior lines for further development. Cultivars successfully commercialized by Sevita International will increase contract production at a grower level and will garner higher premiums in the market place thereby increasing value and return to Canadian producers.

Milestones

- Incorporation of specific quality traits identified and provided by Japanese researchers and end-users into adapted Canadian soybean germplasm
- Targeting specific varieties to growing areas to maximize production and quality
- Engaging marketplace end-users and buyers in the selection of superior lines at early stages of varietal

development

- Promotion of enhanced traits by Japanese buyers in advance of the release of the varieties with those traits

Table 4. Tests S76C/S77C: Conventional MG0, 1 OAC F7 Special Traits Advanced Yield Trials, 1 YEAR DATA SUMMARY 2012 (S76C - ELORA RS, S77C - WOODSTOCK RS)

VARIETY	TRAIT	YIELD (kg/ha)	DAYS TO MATURITY	LODGE (1 to 5)	HEIGHT (cm)	100 SEED WEIGHT (g)	SEED QUALITY (1 to 5)	OIL (%)	PROTEIN (%)
11S68C-37	Low Linolenic	2531	106	1.1	61	17.7	1.5	21.3	41.1
11S68C-38	Low Linolenic	2430	106	1.0	51	20.1	1.5	20.8	43.6
OAC Wellington	Check	3170	107	1.3	73	19.9	1.5	21.1	44.3
11S68C-06	Tocopherol	2598	107	1.0	55	18.0	1.5	19.6	43.5
OAC Madoc	Check	2438	107	1.0	56	19.0	1.5	21.8	41.9
11S68C-18	Tocopherol	2025	107	1.0	50	17.4	1.8	21.5	41.3
11S68C-22	Tocopherol	2630	108	1.2	66	17.2	1.8	21.1	42.7
DH618	Check	2628	108	1.0	66	17.5	1.5	21.2	42.5
11S68C-16	Tocopherol	2080	108	1.0	51	17.9	1.5	20.1	42.9
OAC Champion	Check	2897	109	1.0	73	17.8	1.5	21.1	43.5
11S68C-41	Low Linolenic	2473	109	1.0	62	17.8	1.8	21.1	41.6
OAC Wallace	Check	3222	110	1.0	71	18.3	1.5	22.9	38.7
11S68C-14	Tocopherol	2768	110	1.1	69	18.6	1.5	20.9	41.8
11S68C-43	Low Linolenic	2715	111	1.1	60	19.7	1.5	21.1	41.0
OAC Purdy	Check	3250	112	1.0	75	17.2	1.5	21.4	42.5
11S68C-27	Lipoxygenase	2481	112	1.1	61	19.4	1.5	20.7	45.4
11S68C-45	High Linoleic	2908	113	1.0	66	16.8	1.5	22.3	40.9
11S68C-23	Tocopherol	3020	114	1.7	87	16.3	1.8	20.7	43.2
11S68C-29	Lipoxygenase	3012	114	1.0	66	22.8	1.5	21.0	44.3
11S68C-28	Lipoxygenase	2343	115	1.1	59	19.5	1.5	20.5	42.8
11S68C-40	Low Linolenic	3116	116	1.0	78	20.4	1.5	20.9	40.0
11S68C-39	Low Linolenic	3337	117	1.0	72	20.0	1.8	21.3	41.7
11S68C-34	Lipoxygenase	3263	117	1.0	74	25.0	1.5	20.7	43.5
11S68C-31	Lipoxygenase	2966	117	1.0	63	23.3	1.5	20.9	43.6
11S68C-42	Low Linolenic	3735	118	1.0	81	18.5	1.5	21.3	39.4
Katrina	Check	3433	118	1.0	78	20.8	1.5	21.1	43.3
11S68C-44	High Linoleic	2980	118	1.2	77	15.8	1.5	22.1	40.2
11S68C-36	Low Linolenic	3010	119	1.1	65	19.0	2.0	21.1	42.7
11S68C-32	Lipoxygenase	3445	120	1.0	69	24.2	1.5	20.5	43.9
OAC Perth	Check	3353	120	1.0	69	19.1	1.5	21.5	41.9
OAC Prodigy	Check	3293	120	1.0	71	20.5	1.5	21.2	41.3
11S68C-46	High Linoleic	3418	121	1.3	75	14.9	1.8	22.2	39.4
11S68C-10	Tocopherol	2959	122	1.3	71	20.3	1.8	21.2	43.2
GRAND MEAN		2922	113	1.1	67	19.2	1.6	21.1	42.2
CHECK MEAN		3076	112	1.0	70	18.9	1.5	21.4	42.2
No. of Locations		2	2	2	2	2	2	2	2
Total No. of Reps		4	4	4	4	2	2	2	2

Lodging at maturity: (1-5) 1=standing, 5=flat
Seed quality: (1-5) 1=exceptional, 5=poor

Methodology – University of Guelph

Of the original 70 cultivars with high tocopherol (Vitamin E), zero lipoxygenase, low linolenic and high linoleic traits tested in preliminary (F6) and advanced (F7) yield trials in Ontario in 2011, seven high tocopherol (vitamin E) lines were tested in replicated advanced yield trials in two locations on Ontario in 2012. The maturity range for these lines was between 107 and 122 days and some of them yielded close to the yield of the check varieties.
Additionally, six F7 lines with zero lipoxygenase and low linolenic seeds, which are traits desirable by the food grade industry, have been tested in Advanced Yield Trials in two locations in Ontario in 2012. Several performed very well against the checks with the best one being 11S68C-32, which yielded 3445 kilograms per hectare (kg/ha) compared to the closest checks, OAC Perth (3353kg/h) and OAC Purdy (3250kg/h).
In a special traits soybean private trial, a number of soybean lines with special traits that are of interest to nutraceutical, food grade and other soybean industry uses have been tested. The tests were conducted using selected lines that were mentioned above in two field locations, Elora and Woodstock, ON, in 2012. The results that were obtained are included in Table 4.

Results and Discussion

Based on the above results, Sevita International has selected the two following lines for continued testing and potential commercialization:

1. 11S68C-32 and
2. 11S68C-42

The balance of the lines has been entered into private trials in multiple locations in 2013 for possible release to the soybean industry in 2014.

Introduction - AAFC Harrington

Three basic cultivar evaluation trials were conducted under this project sub-activity in 2012, which were continuation of trials conducted in 2011. Two of the trials were Identity Preserved (IP) Tofu soybean cultivar evaluations and one an IP Natto soybean cultivar evaluation trial.
In 2011, one of the Tofu trials was also utilized to include an indication of the dry down characteristics of the lines in the trial. For 2012, a smaller selection of lines from the 2011 trial was utilized and represented a range of maturity characteristics.
A large head row selection block was included in the study in 2011 to select early generation germplasm which may have potential for PEI and the Atlantic Canadian region in general regarding appropriate maturity characteristics, disease resistance and reasonable general agronomic characteristics. In 2012, by mutual agreement between Sevita International and AAFC Harrington Research Farm, the head row selection block was replaced by an adaptation comparison cultivar evaluation trial of a range of food grade soybean lines/cultivars originating from different breeding programs.
In addition to the trials on germplasm/cultivar evaluation, additional trials on seed and foliar fungicide applications were conducted as part of this project and a project funded through the Atlantic Grains Council.
Following is the summary of the trials conducted during the summer of 2012. The 2012 production year was highlighted by a prolonged period of low precipitation during the months of June through August. Across PEI, there were areas of higher moisture during this period that in others with scattered heavy rainfalls. September through to harvest was a wet period, adding to issues relating to moisture content at harvest and the drying down of the beans. Plots were harvested at maturity, noting that with the number of plots to be harvested it was necessary to harvest as soon as possible, and at higher moisture levels than would normally be preferred.

Methodology – AAFC Harrington

During each season, the plots were monitored on a regular basis for the appearance and severity of any foliar disease or other issues, which may impact the crop responses. In general there were no disease symptoms of any consequence in any plot. There were minor symptoms of root rot pathogens present in the field but these were at very low levels and no plot exhibited any indication of these infections having an effect on crop development. Nodulation in the field was good to excellent.

Methodology - Soybean Dry Down Trial (year 2)

Compared to 2011, where 30 lines where employed, only 10 entries where utilized in the 2012. These entries represented a range of maturities and apparent dry down rates from the 2011 results. To measure the rate of change in moisture content, the experiment was set out as two plots of each entry, when compared to production trials. One plot was used for the agronomic data, while the adjacent plot was destructively sampled during the dry down period, with all pods of ten randomly selected plants per plot being collected and moisture content determined. Once bean formation reached a point where they could be relatively easily removed from the pod, the beans were removed and the total moisture of pods and beans determined along with just the bean moisture level.

Results and Discussion - Soybean Dry Down Trial (year 2)

The basis agronomy data is presented in Table 5. The lines selected for this trial demonstrate a broad spectrum of days to maturity (105 to 111 days after seeding) and in yield potential (1928 to 2501 kg/ha). There was a significant relationship between leaf drop as measured on Sept 11 and days to maturity (P=0.003, R=0.835). While the first leaf drop assessment was significant related to yield (P=0.013, R=0.746) this was not apparent with regards to days to maturity and yield. As expected, the trend was the earlier the line matured or started to mature, the lower the yield.

Table 5. 2012 Soybean cultivar dry down evaluation trial results

Entry No.	Entry	Plant Count (#/m)	Plant Height (cm)	Pod Height (cm)	11-Sep Leaf Drop (0-10)	17-Sep Leaf Drop (0-10)	24-Sep Leaf Drop (0-10)	Lodging (0-10)	Maturity (days)	Yield @13% (kg/ha)	Moisture (%)	100 Seeds (g)
1	DH863	16.7	49.7	6.7	7	10	10	0	107.3	2203	18.48	18.2
2	Savana	20.7	49	5.7	3.3	9.3	10	0	111	2243	18.11	17.9
3	DH420	18.7	52.7	8.7	3.3	9.7	10	0	109.7	2501	18.62	19.6
4	DH121144125	18	44.7	9	6.3	10	10	0	108.7	1975	18.19	18
5	DH121144116	19.3	51	6	8.3	10	10	0	108.7	1928	17.54	16.4
6	DH121144127	29.3	45.3	5.7	9	10	10	0	106.3	1952	18.35	15.9
7	DH121144110	21.3	59.7	11.7	5.7	10	10	0	108	1994	19.22	21.9
8	DH121144114	22	53	9	9	10	10	0	105	1988	18.65	16.8
9	DH401-3	18	49.3	7	7	10	10	0	108	2226	18.51	18.4
10	DH121144105	19.3	49	8.7	6	10	10	0	107.7	2198	18.37	18.9
	SEM	2.083	2.433	1.137	0.454	0.1449	*	*	0.402	49.2	0.1599	0.2648
	LSD (0.05)	6.19	7.23	3.38	1.35	ns	*	*	1.2	146.2	0.475	0.79

Pod height = The height from the ground to the first pod formation
Lodging: 1= standing 10=flatLeaf drop: 0=no leaf drop, 10 complete leaf drop

The dry down characteristics of the lines are presented in Table 6 and represents the whole pod moisture content and, for later stages in crop maturity, both whole pod and the bean moisture levels.

Figures 9 and 10 provide a visual representation of the moisture levels of pods and beans, respectively, over time.

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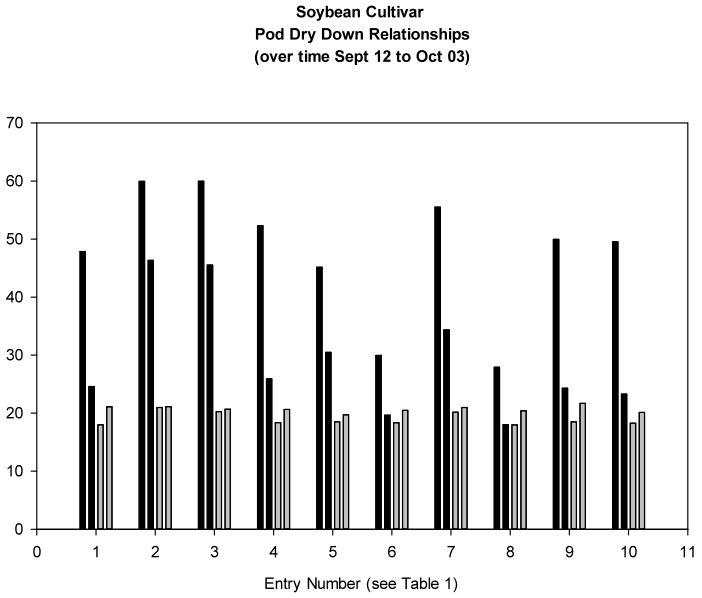
Research Project Final Report

Table 6. 2012 Soybean Cultivar Dry Down Evaluation Trial - Pod and bean moisture levels over time

		Pod					Bean		
Entry	Entry	Moisture					Moisture		
No.		12-Sep	19-Sep	26-Sep	03-Oct		26-Sep	03-Oct	11-Oct
		(%)	(%)	(%)	(%)		(%) *	(%)	(%)
1	DH863	47.9	24.6	18	21.1		18	18.7	18.5
2	Savana	60	46.3	21	21.1		19.7	17.4	18.1
3	DH420	60	45.5	20.3	20.7		17.2	17.9	18.6
4	DH121144125	52.3	25.9	18.4	20.6		18	18.2	18.2
5	DH121144116	45.2	30.5	18.5	19.7		16.7	17.2	17.5
6	DH121144127	30	19.6	18.3	20.5		17.1	18.6	18.3
7	DH121144110	55.5	34.3	20.2	21		20.2	18.3	19.2
8	DH121144114	27.9	18	18	20.4		17.3	18	18.7
9	DH401-3	50	24.3	18.5	21.7		17.7	18.3	18.5
10	DH121144105	49.5	23.3	18.3	20.1		18.4	17.6	18.4
	SEM	1.089	1.519	0.406	0.422		*	0.506	0.1599
	LSD (0.05)	3.24	4.51	1.21	1.25		*	ns	0.48

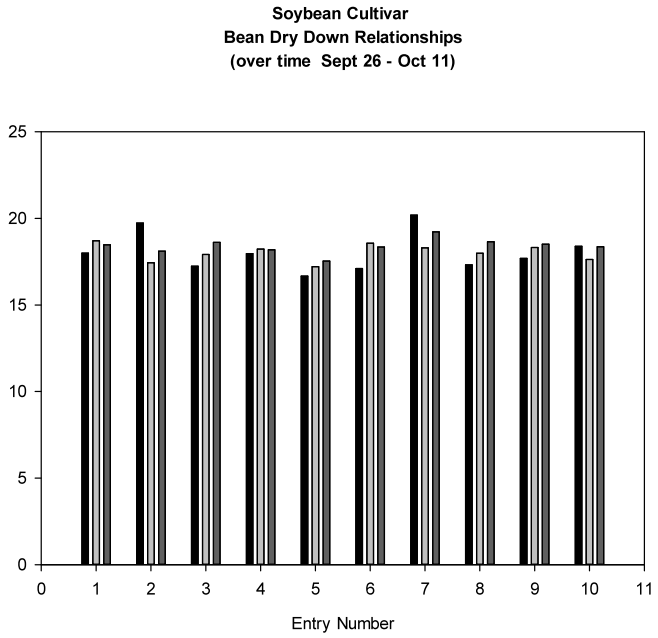
* One replicate only shelled/evaluated



Entries :

1	DH863	6	DH121144127
2	Savana	7	DH121144110
3	DH420	8	DH121122114
4	DH121144125	9	DH401-3
5	DH121144116	10	DH121144105

Figure 9: Pod Dry Down Relationships between different Cultivars (moisture dates: September 12, 19, 26 and October 3)



Entries :

1	DH863	6	DH121144127
2	Savana	7	DH121144110
3	DH420	8	DH121122114
4	DH121144125	9	DH401-3
5	DH121144116	10	DH121144105

Figure 10: Bean Dry Down Relationships between different Cultivars (moisture dates: September 12 and October 3, 11)

The earlier the maturity date, the lower the moisture content early on in the evaluation timeline. It can be noted that the lines all exhibited similar dry down characteristics, with longer maturing lines drying down slower at earlier stages, as would be expected.

Methodology - Tofu Soybean Trial #1

A total of 30 entries were evaluated in a three replicate trial, and agronomic data is presented in Table 7. During the season the plots were monitored on a regular basis for the appearance and severity of any foliar disease or other issues which may impact upon the crop responses.

There were few significant differences among what could be considered the four check type lines, in particular DH401, DH407, DH413 and DH863. Line DH407 did appear to be an earlier genotype based on degree of leaf drop at the first assessment date (Sept 11). This early start to senescence was not reflected in a dramatically earlier maturing line, maturing only about two days earlier when compared to the other check lines.

Table 7: 2012 tofu soybean cultivar evaluation trial

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Entry No.	Entry	Plant count (1m)	Plant Height (cm)	Pod Height (cm)	Leaf Drop Sept 11 (0-10)	Leaf Drop Sept 17 (0-10)	Leaf Drop Sept 24 (0-10)	Lodging (0-9)	Maturity after seeding (days)	Yield @ 13% (kg/ha)	Moisture at Harvest (%)
1	HS05-09/H-5	24	55.7	11	5.7	10	10	0	109	2319	17.31
2	HS05-18/1b/1/1/1/18c	21.7	47	6.7	7.7	10	10	0	108	2232	16.68
3	HS05-27/1/1/1/17b	24	61	12.7	6.3	10	10	0	108	2017	18.06
4	HS05-34/1b/1/1/1/6a	21.3	54	8.7	4.7	10	10	0	109	2205	16.85
5	HS05-37a/1b/1/1/1/7a	23.7	59.7	7.7	2.3	9.3	10	0	110	2360	17.61
6	HS06-20/1/1/1/1/17b	22.3	53	11.3	7	10	10	0	107	2159	16.83
7	HS06-22/1/1/1/1/37c	24.3	59.3	8	6.3	9.7	10	0	109	2252	16.72
8	HS06-22/1/27SP/1b	22.3	68.3	12.7	3.7	10	10	0	111	2248	16.77
9	HS06-28/1/1/1/1/3a	20.3	49.7	12.3	9	10	10	0	107	2210	16.7
10	HS06-29/1/1/1/1/34c	21.7	55	10.3	8	10	10	0	107	2263	16.7
11	HS06-31/1/1/1/1/10b	24.3	55.7	9.3	6	10	10	0	108	2336	17.34
12	HS06-31/1/1/1/1/16a	15.7	50.3	9.3	5.7	10	10	0	109	2429	17.29
13	HS06-31/1/1/1/1/5b	21.3	54	9.3	6.7	10	10	0	108	2294	17.48
14	HS06-61/1/1/1/7c	26	59.3	6.7	5.7	10	10	0	109	2386	17.37
15	HS06-63/1/1/1/9c	25.3	59.7	11.7	6.7	10	10	0	109	2376	17.42
16	HS06-64/1/1/1/1b	22.7	59.7	8.7	9	10	10	0	108	2044	17.08
17	HS06-64/1/1/1/5b	27	58	7.3	9	9.7	10	0	107	2055	16.83
18	HS06-66/1/1/1/23b	25	63	7.7	8.3	10	10	0	108	2065	16.47
19	HS06-66/1/1/1/6c	24.7	56.3	9.3	9.7	10	10	0	101	2060	17.07
20	HS05-28/1b/1/1/1/39c	19.7	51.3	10.7	4.3	10	10	0	109	2060	17.1
21	HS06-60/1/1/1/6b	22.3	54	12	3	10	10	0	110	2255	17.39
22	HS05-13a/1b/1/1/1/5b	30	56.3	7.7	6.3	10	10	0	108	2092	17.44
23	HS05-02/a/1/1/1/30RILS	22.7	56.3	10.7	6	10	10	0	108	2333	17.28
24	HS06-22/1/1/1/1/20b	22	63.3	11	1	9	10	0	113	2277	17.6
25	HS05-28/1/1/1/1a	22.3	58.3	9.3	0.7	9	10	0	111	2239	17.67
26	HS05-35/1b/1/1/1/3a	22	53.7	10	0.7	9.3	10	0	111	2396	17.7
27	DH413	25.7	52.3	8	4.7	10	10	0	109	2246	17.3
28	DH863	23.7	52.7	8	5.7	10	10	0	108	2312	17.23
29	DH407	20.3	54	8.3	8	10	10	0	107	2331	17.49
30	DH401	24	54.7	9.3	5	9.7	10	0	109	2333	17.35
	SEM	2.376	2.906	1.749	0.627	0.1725	0	0	0.714	55.2	0.1412
	LSD (0.05)	6.73	8.23	4.95	1.78	0.49	ns	ns	2	156.2	0.4

Pod height = The height from the ground to the first pod formation
Lodging: 1= standing 9=flat
Leaf drop: 0=no leaf drop, 10 complete leaf drop

Over all, there was a significant correlation between degree of leaf drop on September 11 and the days to maturity (R=0.814). Figure 11 outlines this relationship for all entries in the trial and it can be noted that there was notable

outlier to this trend. Line HS06-66/1/1/1/6c (Entry 19) was a late line in terms of starting leaf drop, but was the earliest of the lines in trial by six to eleven days. This rapid rate of maturity was not reflected in a yield response, which would make it ideal for the normally shorter season climatic situation normally experienced in the region. However, this line may still have potential in late seeding situations or where the season is unduly short. The latter cannot be forecasted, but should be considered over a large number of years.

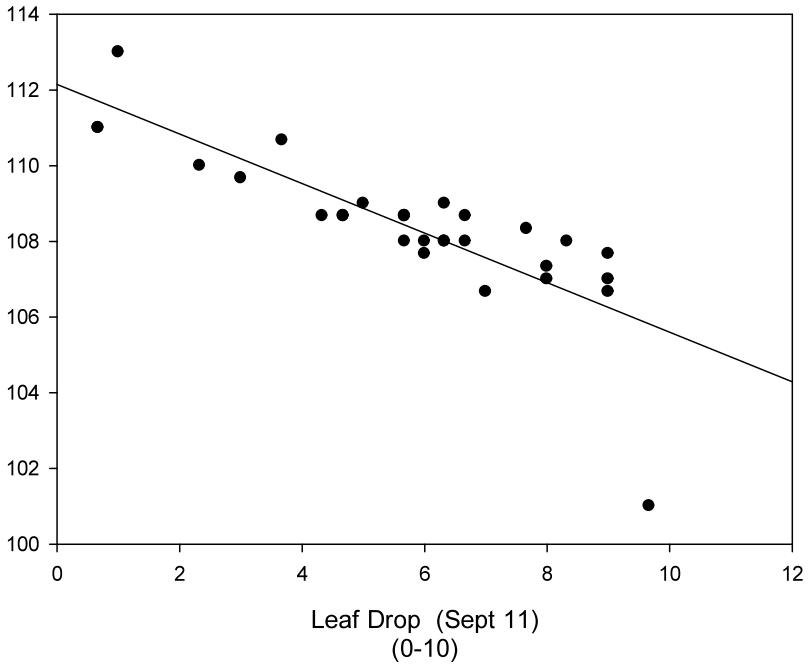


Figure 11: Maturity relationships in tofu trial (Sevita #65), Early leaf drop versus days to maturity
0 = no leaf drop, 10 = complete leaf drop

Samples from the trial were forwarded to Sevita International's facilities in Ontario for evaluations (Table 8).

Table 8. Summary of Sevita International evaluations of AAFC Harrington tofu soybean trial #1

Entry	Name	Yield (Bushel/ acre)	Yield (kilogram/ hectare)	Rank	Predicted Yield (%)	Days to Maturity	Plant Height (cm)	Population Score (1-9)	Pod Height (cm)	100 Seed Weight(g)	Oil (%)	Protein (%)	Colour Flower	Colour Pubescence	Colour Hilum	Colour Seed	Seed Lustre	Seed Shape
19	HS06-66/1/1/1/6c	30.2	2011	30	99.6	101	59	8.1	9.3	16.0	22.4	34.5	P	G	Y	Y	D	FR
29	**PSX12C51S**	35.2	2342	7	106.8	107	56	6.9	8.3	17.2	21.4	32.8	P	T	ly	ly-Y	D	FE
10	HS06-29/1/1/1/1/34c	33.7	2243	18	102.3	107	56	7.2	10.3	19.2	21.9	33.4	P	T	ly	Y		
9	HS06-28/1/1/1/1/3a	33.0	2198	20	100.2	107	49	6.8	12.4	21.4	21.7	34.6	P	T	ly	Y	D	FE
6	HS06-20/1/1/1/1/17b	32.3	2151	23	98.0	107	53	7.4	11.4	11.9	21.9	31.2	P	G	Y	Y	D	FR
17	HS06-64/1/1/1/5b	31.1	2073	27	94.4	107	56	8.6	7.3	14.9	22.3	33	P	T	ly-Y	Y	D	FR
11	HS06-31/1/1/1/1/10b	35.6	2374	3	106.6	108	56	8.1	9.3	17.1	21.7	33.3	P	T	ly	ly-Y	D	FE
23	HS05-02/a/1/1/1/30RILS	34.6	2305	11	103.6	108	57	7.7	10.7	16.9	22.2	32.3			ly	ly-Y	D	FE
13	HS06-31/1/1/1/1/5b	34.5	2297	12	103.3	108	53	6.9	9.4	16.7	21.8	33.3	P	T	ly	ly-Y	D	FE
28	**DH863**	34.3	2290	13	102.8	108	50	7.8	8.0	17.2	22.8	32.7	P	T	ly	ly-Y	D	FE
2	HS05-18/1b/1/1/1/18c	32.9	2190	21	98.6	108	48	7.1	6.6	16.3	22.2	32.4	P	T-G	ly	Y	D	FR
22	HS05-13a/1b/1/1/1/5b	31.4	2091	25	94.1	108	57	9.8	7.7	16.4	21.1	34.4			ly	ly-Y	D	FE
16	HS06-64/1/1/1/1b	31.2	2080	26	93.5	108	61	7.4	8.6	14.7	22.6	32.6	P	T-G	ly-Y	Y	D	FR
18	HS06-66/1/1/1/23b	31.1	2072	28	93.2	108	62	8.1	7.7	17.1	22.5	32.9	P	T	ly-Y	Y	D	FR
3	HS05-27/1/1/1/1/7b	30.6	2039	29	91.7	108	61	8.0	12.7	19.9	20.7	32.8	P	T	Y	ly-Y	D	FE
12	HS06-31/1/1/1/1/16a	36.8	2452	1	108.8	109	50	5.0	9.4	16.8	21	34.1	P	T	ly	ly-Y	D	FE
15	HS06-63/1/1/1/9c	35.7	2378	2	105.6	109	60	8.7	11.6	16.0	22.5	32.5	P	T	ly-Y	ly-Y	D	FE
14	HS06-61/1/1/1/7c	35.2	2345	6	104.1	109	61	8.5	6.6	17.1	21.5	33.6	P	T	ly	ly-Y	D	FE
30	**DH401**	35.2	2347	5	104.1	109	56	8.1	9.3	17.3	21	33.9	P	T	ly	ly-Y	D	FE
1	HS05-09/H-5	34.8	2322	10	102.9	109	56	8.0	11.1	16.7	21.5	33.1	P	T	ly	ly-Y	D	FE
7	HS06-22/1/1/1/1/37c	33.9	2259	15	100.2	109	58	8.2	8.0	14.9	22.5	33.1	P	T	ly	Y-BR	D	FR
27	**PSX12C41S**	33.7	2249	16	99.7	109	53	8.6	8.0	16.5	20.8	34	P	T	ly	ly-Y	D	FE
4	HS05-34/1b/1/1/1/6a	32.8	2189	22	97.0	109	54	7.0	8.7	16.8	22.2	32.9	P	T	ly	ly-Y	D	FE
20	HS05-28/1b/1/1/1/39c	31.7	2115	24	93.7	109	50	6.3	10.7	16.0	22.4	32.8			ly-Y	ly-Y	D	FE
5	HS05-37a/1b/1/1/1/7a	34.8	2322	9	101.6	110	59	7.9	7.7	18.2	21.3	36.6	P	T	ly	ly-Y	D	FE
21	HS06-60/1/1/1/6b	33.6	2240	19	98.1	110	53	7.4	12.0	17.3	22	33.3	P	T	ly	ly-Y	D	FE
26	HS05-35/1b/1/1/1/3a	35.2	2348	4	101.5	111	53	7.3	10.0	18.9	21.4	34.4			ly	ly-Y	D	FE
8	HS06-22/1/27SP/1b	34.1	2275	14	98.3	111	68	7.2	12.6	17.1	23.2	32	P	T	ly	ly-Y	D	FE
25	HS05-28/1/1/1/1a	33.7	2248	17	97.1	111	57	7.6	9.3	16.0	22	32.4			ly	ly-Y	D	FE
24	HS06-22/1/1/1/1/20b	35.1	2341	8	98.7	113	62	6.9	11.0	17.5	21.2	34.5			ly	ly-Y	D	FE
	GRAND MEAN	33.6	2239			108	56	7.6	9.5									
	CHECK MEAN	**34.6**	**2307**			**108**	**54**	**7.9**	**8.4**									
	Coefficient of Variation	3.70	3.70			1.14	8.09	17.51	31.81									
	Least Significant Difference	2.45	163.64			2.41	8.96	2.63	5.97									
	Standard Error of the Difference	0.93	62.19			1.01	3.43	1.04	2.46									
	R-Square	0.90	0.90			0.80	0.76	0.61	0.52									
	Alpha level	0.01	0.01			0.01	0.01	0.01	0.01									
	Method	ALS	ALS			ALS	ALS	ALS	ALS									
	Number of Repetitions	3	3			3	3	3	3									

Yield adjusted to 13% moisture
Visual score at maturity: (1-9) 1= poor, 9=very good
Population score: (1-10) 1=10%, 10=100%
Lodging at maturity: (1-5) 1=standing, 5=flat
Flower colour: P=purple, W=white, M=mixed
Pubescence colour: T=tawny, G=grey, LT=light tawny, M=mix
Leaf shape: O=oval, L=lanceolate(Narrow), OL,LO=mixed

Tofu Soybean Trial #2 (Sevita Trial #66)

A total of 30 entries were evaluated in a three replicate trial, and agronomic data is presented in Table 9. During the season the plots were monitored on a regular basis for the appearance and severity of any foliar disease or other issues which may impact upon the crop responses.

As with the previous trial (Sevita Trial #65) there was a significant linear regression relationship (R=0.776) between early leaf drop and maturity Figure 12. One line however did deviated significantly from the general trend of the other lines, in particular DH863 had a much higher leaf drop at the first rating (Sept 11), where leaf drop was 8.3 out of 10, where the remaining lines barely had any leaf drop at all (Table 9). It also matured at least six days earlier than the next earliest line. The later maturity in the lines in this trial was reflected in a slightly higher moisture content at harvest, averaging 19.6%. Even though the moisture was high, the large number of plots and the wet nature of the fall harvest period of 2012 meant harvesting as soon as possible.

AAFC RESEARCH BRANCH
Research Project Final Report

Table 9. 2012 tofu soybean cultivar evaluation trial (Sevita Trial #66)

Entry No.	Entry	Plant count (1m)	Plant Height (cm)	Pod Height (cm)	Leaf Drop Sept 11 (0-10)	Leaf Drop Sept 17 (0-10)	Leaf Drop Sept 24 (0-10)	Lodging (0-9)	Maturity after seeding (days)	Yield @ 13% moisture (kg/ha)	Moisture at Harvest (%)
1	HS06-31/1/1/1/1/7c	21.3	59	12.3	0.3	7.3	10	0	115	2510	19.86
2	HS06-28/1/1a/1/1/8a	22	56.3	11.3	0	2.7	9.3	0	116	2788	19.43
3	HS05-18/1b/1/1/1/1a	18.3	60.7	13	0	6	10	0	116	2789	18.83
4	HS06-21/1/1/1/1/17c	21.7	60	12	0	3.3	10	0.7	116	2526	20.43
5	HS05-06/a/1/1/1/14	25.3	63.3	5.7	0	5.7	10	0	115	2414	19.55
6	HS05-06/a/1/1/1/31	16	60.3	12	0	8	10	0	113	2647	18.14
7	HSG07-2-1	24.7	55	10	0	2.3	10	0	117	2610	20.27
8	HSG07-209-1	25	48.3	9.3	0	2.7	9.7	0.3	118	2607	20.31
9	HS05-06a/1b/1/1/1/26a	20.3	59.3	10.3	0	5.3	10	0	117	2434	20.01
10	HS05-27/1/1/1/1a	26.3	53.7	10.3	0	6.3	10	0	116	2433	19.32
11	HS06-22/1/1/1/1/15c	28	69.7	11.7	0	3.7	10	1.3	118	2627	19.69
12	HS06-30/1/1/1/1/17c	27	57.7	12.7	0	7	10	0	115	2489	18.77
13	HSG07-208-1	21.3	56	7.3	0	6.7	10	0.3	117	2488	21.7
14	HS05-09/a/1/1/1/8a	20.7	66	12.3	0	6.3	10	0	115	2602	19.17
15	HS05-09/a/1/1/1/9c	25.3	57	11	0	4	10	1	119	2663	18.62
16	HS05-10a/1b/1/1/1/16a	21.3	64.3	11.7	0	5	10	0	117	2748	20.32
17	HSG07-2-4	23.3	59.7	6.3	0	6.3	10	0.7	117	2675	20.25
18	HS06-22/1/1/1/1/3a	20.7	60.3	13	0	3.7	9.7	3.3	120	2660	18.99
19	HSG07-216-1	21.3	48	6	0	6.3	10	0	116	2465	20.19
20	HS05-35/1/1/1/17b	17.3	64	7.7	0	7	10	0	115	2508	19.5
21	HS05-35/1b/1/1/1/3a	19	59.7	8.7	0.3	8.7	10	0	113	2521	19.74
22	HS06-21/1/9SP/1b	20.3	57.7	11	0	3	10	0	117	2709	18.7
23	Savanna	21.3	60.3	6.7	0	8.3	10	0	114	2578	19.07
24	HS06-22/1/1/1/1/3a	22.7	61.7	14.7	0	4	10	0.7	119	2577	19.84
25	HS06-30/1/1/1/1/5c	22.3	61.3	11	0	4.7	10	1	117	2458	19.69
26	OAC Sunny	23	56.7	8.7	0	3	9.3	0	118	2752	19.68
27	DH863	24.3	54.7	10.3	8.3	9.7	10	0	107	2528	19.53
28	S05-T6	19	57.3	8.7	0	5	10	0	116	2652	18.87
29	DH618	26	60.3	11	0.3	7.7	10	0	114	2727	19.95
30	DH408	17.3	60	12.7	0	3.7	9.7	0	118	2646	18.2
	SEM	2.577	2.991	1.609	0.1201	0.562	0.1351	0.4029	0.976	61.6	0.3568
	LSD (0.05)	7.29	8.47	4.56	0.34	1.59	0.38	1.14	2.8	174.3	1.01

Pod HEIGHT = The height from the ground to the first pod formation
Leaf drop 0= no leaf drop, 10= complete leaf drop, lodging 1= standing, 9= flat.

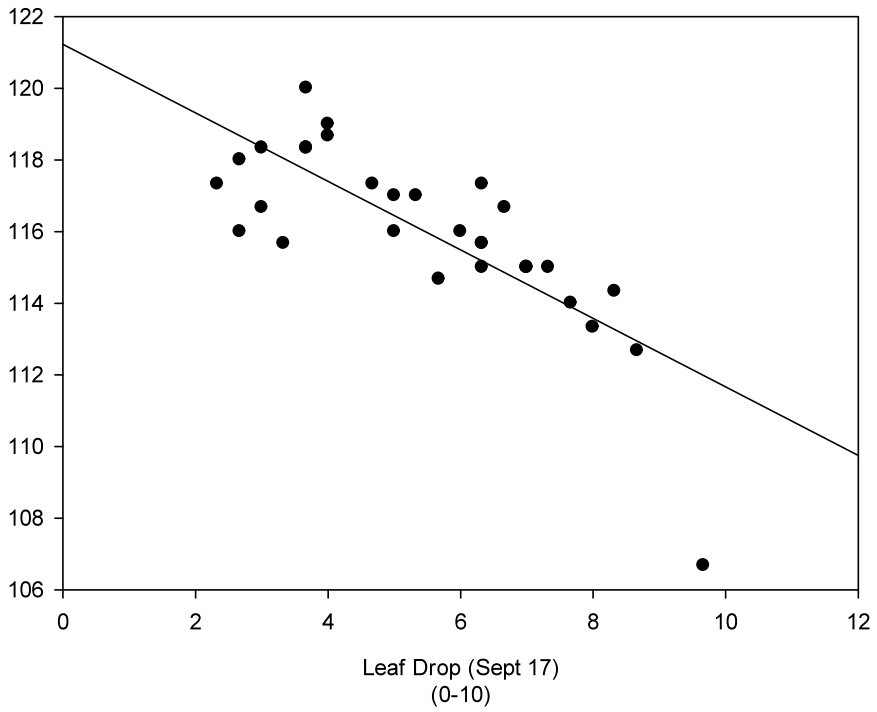


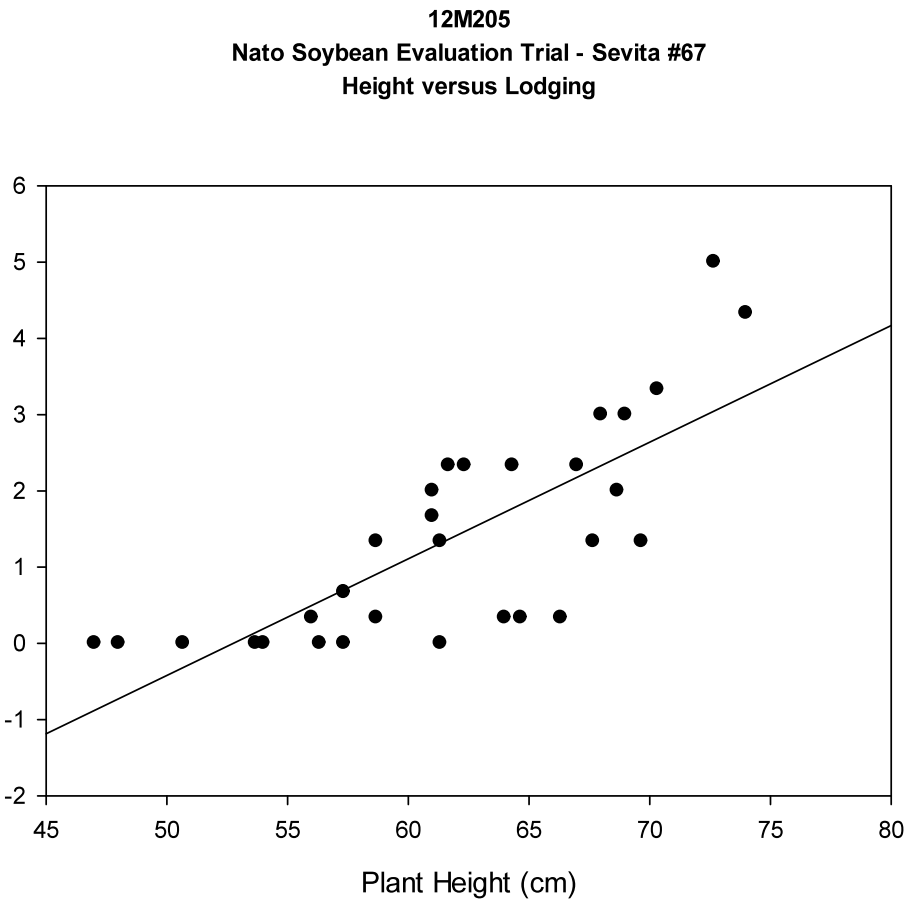
Figure 12: Maturity relationship in Sevita Trial # 66, Early leaf drop versus days to maturity
Leaf drop 0=no leaf drop, 10=complete lead drop

Several lines in this trial exhibited significant lodging, noting HS06-22/1/1/1/1/3a in particular had relatively poor lodging resistance, especially when lodging was not in general an issue in the plot area in 2012. Lines in this trial were later in maturity when compared to those in Sevita Trial #65 by an average of eight days, with one line (HS06-22/1/1/1/1/3a) being 12 days later. This later maturity did appear to result in an average yield increase of over 10% over the mean of the shorter season trial, with HS05-18/1b/1/1/1/1a and HS06-28/1/1a/1/1/8a having the best increase within the trial (10% over the common check). A late maturity was not a production issue in 2012 as the first frost was late in the fall. Thus, the long term advantage of longer season lines remains to be determined. The earlier maturing cultivar, DH863, still had excellent yield production.

Natto Soybean Trial (Sevita Trial #67)

A total of 30 entries were evaluated in a three replicate trial, and agronomic data is presented in Table 9. During the season, the plots were monitored on a regular basis for the appearance and severity of any foliar disease or other issues which may impact upon the crop responses.

Figure 13: Natto evaluation trial, height versus lodging



In general, there were no disease symptoms of any consequence in any plot. There were minor symptoms of root rot pathogens present in the field, but these were at very low levels and no plot exhibited any indication of these infections having an effect on crop development. Nodulation in the field was good to excellent.

As with other trials in 2012, there was a significant relationship between leaf drop and maturity, but there were no notable outlining lines in the trial, where the relationship apparent in other lines did not hold; in particular, any lines with a later leaf drop, but early maturity. The linear regressions were significant (P=0.05) at both September 11 and 17 ratings with R=0.795 and R=0.882, respectively. While there was a significant regression between maturity and yield (at 13% moisture), this was relatively weak at R=0.373.

Compared to the other trials in this project and with other soybean trials conducted in the field, in other projects and in the Atlantic regional trials for feed grade soybeans, lodging in the natto lines was more of an issue with a couple of lines where lodging was as high as 5.0 and 4.3 (0-9 scale where 0 represents no lodging) for DH710 and HS06-02/1/1/1/1/21natto, respectively. As expected, there was a significant (P=0.05) linear regression between plant height and lodging, R=0.768 (Figure 13).

From a yield stand point, there were no lines with superior yield to the check cultivar DH710. However, line DH710 is a late maturing genotype and as such there were a number of lines which could be considered as advantageous to the region, where earlier maturity occurred and yields were equal to or slightly superior to the other check cultivars such as DH3604 and DH333. Line HS06-02/1/1/1/1/14nattoc was one, which had a potentially good level of earliness, lodging resistance and yield response; as did HS06-04/1/1/1/1/17nattoc.

Head to Head Soybean Trial (Sevita Trial #68)

A total of 30 entries were evaluated in a three replicate trial, and agronomic data is presented in Table 10. During the season the plots were monitored on a regular basis for the appearance and severity of any foliar disease or other issues which may impact upon the crop responses.

In general, there were no disease symptoms of any consequence in any plot. There were minor symptoms of root rot pathogens present in the field, but these were at very low levels and no plot exhibited any indication of these infections having an effect on crop development. Nodulation in the field was good to excellent.

While the lines in this trial were relatively longer seasoned, several of the entries (greater than 119 days to maturity) exhibited excellent yield response, such as OAC Wallace and DH710 at 3152 and 2980 kg/ha, respectively. However, line DH710 was a very tall entry which was reflected in a relatively high susceptibility to lodging. There was a significant linear relationship between days to maturity and yield (P=<0.001, R=0.696). This noted, there was one major outlier, line DH3604, which was low yielding with mid-range maturity and very small seed. However, the entry is also a natto line, where lower yields and seed size are expected when compared to tofu type lines.

AAFC RESEARCH BRANCH
Research Project Final Report

Table 10. 2012 Soybean Head to Head Trial (Sevita Trial #68)

Entry No.	Entry	Plant count (1m)	Plant Height (cm)	Pod Height (cm)	Leaf Drop Sept 11 (0-10)	Leaf Drop Sept 17 (0-10)	Leaf Drop Sept 24 (0-10)	Lodging (0-9)	Maturity (days)	Yield @ 13% Moisture (kg/ha)	Moisture at Harvest (%)	100 Seed (g)
1	DH413	17.3	56.7	10.7	5.7	10.0	10.0	0.0	107	2555	17.36	20.6
2	Prypiat	24.3	59.0	6.0	10.0	10.0	10.0	0.7	101	2161	17.60	16.3
3	DH863	24.3	61.7	12.3	7.3	10.0	10.0	0.0	107	2610	17.52	20.2
4	DH407	21.3	59.0	8.0	3.0	9.7	10.0	0.0	109	2558	17.49	20.5
5	DH401	25.0	56.7	6.3	6.7	10.0	10.0	0.0	107	2484	17.54	20.6
6	DH404	24.7	57.0	7.7	5.7	10.0	10.0	0.0	108	2533	17.43	20.5
7	DH401-3	25.7	58.3	8.0	6.7	10.0	10.0	0.0	107	2483	17.50	19.8
8	DH508	26.3	54.3	9.3	1.3	9.3	10.0	0.0	111	2526	16.70	21.2
9	OT9814	21.7	55.0	9.3	7.0	10.0	10.0	0.0	106	2466	17.47	19.7
10	FILLER1	18.7	58.0	8.3	5.0	10.0	10.0	0.0	109	2574	17.28	20.2
11	FILLER2	23.3	62.3	9.3	5.3	10.0	10.0	0.0	108	2540	16.76	19.2
12	FILLER3	21.3	62.0	7.7	5.7	10.0	10.0	0.0	107	2492	17.26	21.1
13	DH420	22.0	60.7	10.0	1.3	8.7	10.0	0.3	112	2662	16.95	22.0
14	DH618	22.7	62.0	10.3	1.3	8.7	10.0	0.3	112	2751	17.27	19.1
15	DH401-2	21.3	62.7	14.0	0.0	6.0	10.0	0.7	115	2827	16.31	21.1
16	Savanna	24.3	63.3	8.0	0.0	9.0	10.0	0.0	112	2695	17.00	20.8
17	DH5164-2SCN	23.7	60.3	8.7	0.3	8.0	10.0	0.0	113	2820	16.76	17.5
18	DH6169	24.3	68.7	11.0	0.0	6.3	10.0	0.0	116	2581	15.88	17.3
19	DH3604	22.7	70.7	12.3	0.0	6.7	10.0	1.0	112	2091	16.52	7.7
20	KASSIDY	18.0	63.7	6.0	1.0	9.7	10.0	0.0	111	2526	17.32	19.6
21	OAC Champion	24.3	68.3	8.7	0.7	8.3	10.0	0.7	113	2818	16.73	19.6
22	DH408	24.0	61.3	11.3	0.0	5.3	10.0	0.0	116	2631	16.05	24.2
23	DH506	22.3	67.7	19.7	0.0	2.3	9.3	2.3	119	2981	16.41	21.2
24	DH5164-1SCN	23.0	69.7	9.3	0.0	2.7	10.0	0.0	117	2631	15.71	15.4
25	OAC Sunny	23.7	69.3	8.0	0.0	3.7	10.0	0.0	120	2980	16.28	18.9
26	PRO275	23.0	56.3	10.0	0.0	6.3	10.0	0.0	115	2734	15.58	19.4
27	DH710	23.0	84.7	11.0	0.0	2.3	9.7	3.7	122	2665	15.68	11.7
28	S05-T6	20.7	59.7	10.0	0.0	7.3	10.0	0.0	114	2661	16.05	21.7
29	MADISON	20.3	61.7	6.3	0.0	3.0	10.0	1.3	119	2810	15.80	18.6
30	OAC Wallace	22.7	65.3	12.0	0.0	2.7	9.7	1.7	119	3152	15.54	18.3
	SEM	2.311	3.181	1.583	0.3861	0.4293	0.103	0.308	0.928	80.9	0.2012	0.3512
	LSD (0.05)	ns	9.00	4.48	1.09	1.22	0.29	0.87	2.6	229.0	0.570	0.99

Pod HEIGHT = The height from the ground to the first pod formation
Leaf drop 0= no leaf drop, 10= complete leaf drop, lodging 1= standing, 9= flat.

Sub-activity 1.3

Literature Review and Introduction

Soybean is a key species used by the nutraceutical and functional food industries as it contains several phytochemicals with putative health benefits (Helzlsouer et al., 2000; Faraj and Vasanthan, 2004). It contains high levels of tocopherols which have antioxidant properties. Tocopherols exist in four forms (i.e., α, β, γ, and δ) of which γ-tocopherol is found in greatest concentration in soybean seeds. However, α-tocopherol has the greatest antioxidant activity, and dietary reference intake for vitamin E (i.e. 15 mg day-1) is currently based solely on α-tocopherol (Institute of Medicine, 2000). Other forms do not contribute toward meeting vitamin E requirements because they are not converted to α-tocopherol and are poorly recognized by the α-tocopherol transfer protein in the liver (Institute of Medicine, 2000). If most interest for tocopherol resides in α-tocopherol, being the primary precursor to vitamin E, certain health properties have also been attributed to other tocopherol forms and interest for these remains (Constantinou et al., 2008). It has been suggested that all tocopherols could play a role in cardiovascular diseases and cancer prevention, however such properties remains to be demonstrated.

Factors affecting soybean tocopherol concentrations remain poorly researched, although data to date suggest that they are both genetically and environmentally determined (Ujiie et al., 2005; Scherder et al., 2006; Carrão-Panizzi and Erhan, 2007; Britz et al., 2008). A study recently conducted by Seguin et al. (2009) reported large variation among twenty genotypes for α- tocopherol, a relatively high stability of genotypes performance across six environments in southwestern Quebec, and a lack of negative correlation with seed yield, 100-seed weight, crude protein and oil concentrations. Information on the relation of tocopherol concentrations to other important value added traits such as isoflavones, saponins, lutein, and sugar content remains limited.

Observations from prior studies suggest that selection for high α-tocopherol is possible. The relative stability observed in cultivar performance across environments will also help in the development of functional foods, which requires consistency in nutraceutical compounds concentration. This initial work suggested the feasibility of initiating a selection and breeding program for increased soybean α-tocopherol to develop cultivars that could be used for specific uses by the nutraceutical industry. There is, however, still limited information of the overall stability of tocopherols concentration in soybean especially those specifically developed for the food-grade market.

Objectives

- Short-term objective 1. Determine tocopherols variation and stability among twenty food grade soybean genotypes grown in Ontario and Quebec
- Short-term objective 2. Determine the relationship between tocopherol concentrations and other value-added traits and other important seed characteristics
- Ultimate objective: Identify and release new food-grade soybean cultivars with high and stable tocopherol concentrations and desirable overall agronomic and compositional characteristics.

To meet these objectives two experiments were conducted. For objective 1, a multi-location replicated trial was conducted in 2011 and 2012 at three sites in Eastern Canada with twenty 2800-2900 CHU cultivars or advanced selections from Sevita International. Seed samples were analyzed for tocopherols concentration. Tocopherols data from the six environments were analyzed using various statistical tools, including genotype main effect plus genotype by environment interaction (GGE) biplots and mean tocopherols concentration versus coefficient of variation biplots to assess tocopherol variation and stability among the material evaluated. For objective 2, correlations between several important agronomic, food, and nutraceutical properties were calculated from selected food grade soybean samples originating from the numerous multi-location trials of Sevita International from 2010, 2011, and 2012. These variables were: α-tocopherol, γ-tocopherol, δ-tocopherol, total tocopherol, daidzein, genistein, glycitein, genistein, total isoflavone, lutein, soyasapogenols A, soyasapogenols B, total soyasapogenols, oil, fatty acid, protein, sucrose, stachyose+raffinose, seed yield, seed weight, % emergence, days to flowering, days to maturity, plant height, lodging, white mould incidence. All nutraceutical components were analyzed using wet chemistry.

Performance Indicators

- New lines and cultivars with increased α-tocopherol
- Acquired knowledge on the stability of α-tocopherol across environments in Eastern Canada
- Correlation between tocopherol concentrations and other important characteristics, including other value added traits.

Milestones

- Development of lines and cultivars with increased α-tocopherol.
- Determination of the stability of α-tocopherol across environments in Eastern Canada.
- Determination of the correlation between tocopherol concentrations and other important seed characteristics, including other value added traits.

Materials and Methods - Field plot management

Seeding was done at three sites in both 2011 and 2012 [two in Eastern ON (Inkerman and Ross in 2011, and Inkerman and Kemptville in 2012) and one in Western Quebec (Sainte-Anne-de-Bellevue)] in a prepared seedbed at a rate of 50 plants per metre in the second or third week of May. Each plot consisted of six 5m long rows spaced 18cm apart, with plots assigned to a randomized complete block design with three replicates. A total of twenty soybean genotypes were originally planted with one genotype dropped in the 2012 season (i.e. DH403), while missing samples at one site in 2011 prevented the use of DH5170, giving a total of 18 genotypes included in the final analysis. These included eight released cultivars (i.e. DH4052, DH410SCN, DH4202, DH530, DH6177, DH715L, RCAT Pinehurst, and S12A5) as well as 10 advanced breeding lines (i.e. HS0506a11117, HS05111117, HS0511H24, HS0511H32, HS052411157, HS053211119, HSG0616611111, HSG0616711111, HSG0616711113 and HSG0620311113) for a total of 18 genotypes.
Seeds were inoculated at seeding with a commercial rhizobial inoculant. Plots were fertilized with sufficient phosphorus and potassium during field preparation to support maximal seed yield as recommended by soil tests. Weeds were controlled using herbicides recommended locally for soybean, with manual weeding done later in the season to control weeds that might have not been controlled with herbicides. Plots were harvested with a self-propelled combine when plants from all plots reach maturity (September-October). Upon harvest, seed moisture content was determined and tocopherol concentrations are expressed on a dry matter basis. Upon harvest, sub samples of seeds from each plot were used for laboratory analyses. Seed samples were kept at either room temperature or 4 ºC in a control atmosphere chamber depending on the analytical needs and stability of compounds to be analyzed.

Materials and Methods - Laboratory Analyses

Tocopherols.
Fifty milligrams of finely-ground seeds were weighed in 1.5ml microcentrifuge tubes and supersonicated in 0.5ml of 80% aqueous ethanol (with 5µg/ml of tocopherol as internal standard) for 15 minutes at room temperature. One milliliter of hexane saturated with pyrogallol is added and allowed to stand for 30 minutes at room temperature. This is followed by centrifugation for 10 minutes at 10,000×g, with 0.5ml of the hexane layer (upper phase) then transferred to a new 1.5ml microcentrifuge tube and evaporated overnight. Finally, 0.5ml of 80% aqueous ethanol is added, followed by vortexing at high speed for a few seconds. α-, δ- and γ-tocopherol were separated by HPLC with 20µl of extract used for analyses. Separation was carried out on an Inertsil ODS-3 reverse phase column (5µm, 3.0×250mm; GL Sciences, Japan). The column was maintained at 40°C with a 0.5ml/min flow rate using CH3CN/CH3OH (75:25 v/v) for 25 minutes. Detection is made at 295nm. α-, δ- and γ-tocopherol standards were used to prepare calibration curves. Individual tocopherols were each quantified based on the resulting curves. Tocopherol was used as an internal standard to determine extraction efficiency. Total tocopherol content was calculated by summing up the content of the individual tocopherols. β-tocopherol is another tocopherol found in soybean, but only in very small concentrations, representing usually < 2% of total tocopherol. It is a structural isomer of γ-tocopherol that comigrates with γ-tocopherol on reverse phase columns. Given that, β- and γ-tocopherol have similar calibration curves using standards, β-tocopherol is thus measured as γ-tocopherol, if

present, and therefore total tocopherol was accurately quantified.

Other variables

Dry matter, crude protein (N×6.25) and oil concentrations were determined according to the standard procedures of the Association of Official Analytical Chemists (1990). Sucrose, stachyose and raffinose were all determined using a kit from Megazyme International (Magazyme International Ireland, Ireland) using the procedure provided by the supplier.

Isoflavones were extracted using a modified version of the protocol outlined in AOAC Official Method 2001.10., which relies on saponification of the twelve isoflavones to their glucosidic and aglucone forms. First, 4.6ml of 70% aqueous methanol (with 50µg/ml of apigenin as internal standard) were added to one 100mg of finely ground soybean and supersonicated for 20 minutes at room temperature. Further extraction was carried out by shaking for 60 minutes at 25°C with orbital shaking at a speed of 200rpm after the addition of 300µl of 2M sodium hydroxide. One hundred µl of glacial acetic acid was added to achieve neutralization, which was followed by centrifugation for 10 minutes at 10,000×g; 1.0ml of the supernatant was then transferred to HPLC vials. Isoflavones separation was carried out using a Varian system (Walnut Creek, CA, USA) equipped with a Prostar 210 solvent delivery system, a model 410 autosampler and a Prostar 330 photodiode array detector (PDA). Twenty µl of the extract were used for analyses. Separation was performed on a C18 reversed-phase column (Luna, 5µm, 4.6 × 250mm; Phenomenex, Torrance, CA, USA) with a flow rate of 0.65ml/min, with the column temperature maintained constant at 40°C. Isoflavones were detected at 260nm. Mobile phase solvents, 0.05% phosphoric acid (mobile phase A) and HPLC grade acetonitrile (mobile phase B) were used. Isoflavones elution was carried out using a linear gradient system from 10% solvent B, with no hold time after injection, to 30% solvent B over the course of 60 minutes, followed by a three minute wash with 90% solvent B and 10 minutes for equilibration with 10% of solvent B. Purified isoflavones [daidzein, glycitein, genistein, daidzein, glycitein, and genistein; (Indofine, Hillsborough, NJ, USA)] were used in the preparation of calibration curves. Concentrations of all the isoflavones detected were expressed on dry matter (DM) basis. Isoflavone concentrations were expressed as aglucone equivalent (i.e. daidzein, glycitein and genistein). Total isoflavones concentration was obtained by summing the concentration of individual isoflavones. All data were expressed on an aglucones basis using equations provided in AOAC Official Method 2001.10.

For lutein, twenty-five mg finely-ground seeds were supersonicated in 0.5ml acetone/ethanol (1:1 v/v, with 1µg/ml of Apo-8'-carotenal (trans) as an internal standard) and extracted for 15 minutes at room temperature. This was followed by centrifugation for 10 minutes at 10,000g; the resulting supernatant was transferred to a new 1.5ml tube and then centrifuged at 10,000g for 10 minutes. Twenty ml of the resulting supernatant was subjected to HPLC analysis. Lutein was separated using HPLC a Varian system (Walnut Creek, CA) equipped with a ProStar 210 solvent delivery system, a Model 410 autosampler and a ProStar 330 PDA detector. Separation was carried out on a reverse phase column (5µm, 4.6×250 mm, Phenomenex, USA). The column was maintained at 40°C with a 0.8ml/min flow rate. Mobile phase A consisted of acetonitrile and mobile phase B of ethanol. The gradient was initiated at 25% B and held for 5 minutes and then increased to 75% B in 10 minutes. Afterwards, it was decreased to 25% B in five minutes and held at 25% B for another five minutes. Detection was made at 447nm. Lutein standard (Sigma Aldrich, St. Louis, MO) was used to prepare calibration curves. The content of lutein was then quantified based on the resulting curve.

For soyasapogenol, 0.2 g finely ground soybean powder was dissolved in 20ml of 80% ethanol in a 50ml polypropylene falcon tube. Tubes were consistently shaken at 50°C for two hours. This step was followed by centrifuging the extract at 3000rpm for 10 minutes at room temperature and decanting the clear supernatant. Five ml of the supernatant was dried by speed vacuum overnight. The remaining residue was redissolved in 2ml of 1N HCL in anhydrous methanol. The resuspension was then transferred to a screw-capped glass vial and was subjected to acid hydrolysis at 75°C in a water bath for 2.5 hours and shaking each 20 minutes to release soyasapogenols from soyasaponins. Subsequently, 2ml of the resulting solution was transferred to a 2ml microcentrifuge tube and centrifuged at 13,000rpm at room temperature for 10 minutes. Finally, 300µl of the supernatant was transferred to UPLC sample vial. Soyasapogenols were separated using an Acquity UPLC system equipped with an evaporative light scatting detector (ELSD), which was set to the drift tube temperature of 70°C and the nebulizer nitrogen gas flow was adjusted to 2 ml/min. Separation was carried out on a C18 SB column (1.8µm, 3.0x150mm); the flow rate was 0.4 ml/min with isocratic elution 10 minutes. The mobile phase consisted of acetonitrile:1-propanol:water:0.1 %

acetic acid (80:6:13.9:0.1). Soyasapogenol A and B standards (ChromaDex, Santa Ana, CA) were used to prepare calibration curves. Their content was then quantified based on the resulting curves.
Lipid extraction and methyl ester synthesis were conducted according to O'Fallon et al. (2007). Fatty acid composition of the fatty acid methyl esters was determined by capillary gas chromatography (Varian model 3900 equipped with flame ionization detector at 260°C and model 1177 auto injector) fitted with a fused silica capillary column (CP7489, 100m×0.25mm; Varian, CA, USA). The carrier gas was hydrogen and the flow rate was 0.8ml/min. Injector and detector temperatures were 260°C and the split ratio was 50:1. Column temperature was set at 70°C for 4 minutes and then increased to 130°C at a rate of 12.0°C/min and was maintained for three minutes. It was then increased to 175°C at a rate of 4°C/min and was maintained for 27 minutes. Finally, the temperature was increased to 214°C at a rate of 4°C/min and maintained for 11 minutes and increased to 225°C at a rate of 4°C/min and held for 5.5 minutes.; therefore, total run time was 79.25 minutes. Fatty acids were identified by comparing their retentions times with fatty acid methyl standards (NuCheck Prep Inc., Elysian, MN, USA).

Statistical Analyses

A combined analysis of variance was computed to identify main effects of the environments, genotypes and their interactions on tocopherol concentrations. The analysis of variance was performed using the SAS software (SAS Institute Inc., 2003). Stability analyses were also conducted to determine the stability of genotypes performance for tocopherol concentrations across environments. Values for Francis' and Kannenburg's (1978) mean coefficient of variation stability were computed using SAS. GGE (genotype and genotype x environment) biplots were also used to interpret data using the GGE biplot software (Yan, 2001). Pearson product-moment correlation coefficients were computed based on all the analytical data using the CORR procedure in SAS (SAS Institute, 2003) to describe the relationship between tocopherol concentrations and all other variables measured.

Results and Discussion - Objective 1. Tocopherols variation and stability among food grade soybean cultivars grown in Ontario and Quebec.

The concentration of all tocopherols were affected ($P<0.05$) by genotype, site and genotype by site interactions. Data demonstrate that among the advanced material from Sevita International, there is a large variation in α-tocopherol concentration with values ranging between 7.9 and 38.2µg/g, representing a 382% difference in concentrations across sites and genotypes (Table 11). The variation observed for other tocopherol was smaller being of 95, 48, and 47%, for δ-, γ-and total tocopherol (Tables 12, 13 and 14). The significant genotype by site interactions illustrate that the performance of genotypes varied depending on the cultivars. However, examination of all data using ANOVAs conducted in each individual environment indicates that in most cases, the ranking of genotypes was relatively stable across environments (see Tables 11 to 14). Such observation confirms previous studies conducted with mostly commodity-type soybeans (Seguin et al. 2009). The use of GGE bi-plots reveals that performance of genotypes is strongly positively correlated for δ-, γ- and total tocopherol, but quite distinct for α-tocopherol (Figure 14). This suggests that selection for tocopherols should be conducted either for α-tocopherol or other tocopherols, but could be difficult to achieve for all tocopherol concurrently.
For α-tocopherol, the genotype with the greatest concentration across sites and in five out of six environments was HS0511H24 (entry #9); however, it performed poorly in Sainte-Anne-de-Bellevue in 2011, ranking 12th out of 18 genotypes (Table 11). The instability of this genotype is illustrated in Figure 15 by its position along the blue axis, which indicates variability across environments. The poor performance of this genotype in one specific environment is illustrated by being furthest from SAB11 in the graph. The red axis indicates genotype performance in terms of concentration and entry #9 is, thus, ranking first. Given the poor stability of entry #9, the best performing genotype was entry #10 (i.e. HS0511H32), as it had both high concentrations and stability across environments. Similar results were provided with the graphical representation of Francis and Kannenburg (1978) (data not shown).
The genotype with the greatest total tocopherol concentration across sites and in five out of six environments was DH530 (entry #4); it also ranked second out of 18 genotypes in the sixth environment (Table 12). Although this genotype consistently had very high total tocopherol concentrations, actual concentrations were moderately variable as indicated in Figure 16 by its position along the blue axis, which indicates variability across environments. The most stable genotype was entry #2 (DH410SCN), which ranked in the top four ranks in all individual environments and second across the six environments. This genotype thus appears promising in terms of total

tocopherol concentrations as it had both high concentrations and high stability across environments.

Table 11. Alpha-tocopherol concentrations (micrograms per gram dry matter, µg/g DM) in 18 genotypes grown in six environments in Ontario and Quebec

Table 1. A-tocopherol concentrations (ug/g DM) in 18 genotypes grown in 6 environments in Ontario and Quebec.

Genotype	#	INK11	Rank	ROS11	Rank	SAB11	Rank	INK12	Rank	KEN12	Rank	SAB12	Rank	Avg.	Rank
DH4052	1	19.4	4	16.9	3	18.5	8	23.7	4	25.1	5	21.7	5	20.9	4
DH410GCN	2	9.9	18	8.1	17	14.5	14	15.0	18	15.4	18	9.9	18	12.1	18
DH4202	3	16.3	9	12.1	11	16.4	13	19.7	10	18.5	17	18.3	11	16.9	12
DH530	4	16.1	10	12.7	8	21.8	3	19.7	9	20.1	13	22.9	4	18.9	8
DH6177	5	16.3	8	11.9	12	17.9	10	17.9	11	27.2	3	19.3	10	18.4	9
DH715L	6	13.1	17	10.3	15	14.1	16	15.4	17	20.3	11	16.0	16	14.9	16
HS0506a11117	7	14.7	14	12.6	10	13.9	17	17.2	13	19.5	15	14.3	17	15.4	15
HS05111117	8	18.5	5	16.4	4	19.1	6	25.5	3	26.6	4	20.5	8	21.1	3
HS0511H24	9	28.6	1	21.9	1	16.5	12	31.0	1	38.2	1	30.5	1	27.8	1
HS0511H32	10	25.9	2	17.7	2	23.3	1	28.5	2	28.7	2	27.9	2	25.3	2
HS052411157	11	18.3	6	15.1	6	22.5	2	21.7	7	23.2	8	21.3	6	20.3	6
HS053211119	12	21.5	3	16.1	5	21.1	4	22.5	6	18.8	16	23.7	3	20.6	5
HSG0616611111	13	14.8	13	11.8	13	18.1	9	16.0	16	20.9	10	16.2	15	16.3	14
HSG0616711111	14	13.5	16	9.7	16	13.2	18	16.7	15	19.6	14	16.5	14	14.9	17
HSG0616711113	15	16.5	7	12.6	9	14.1	15	21.4	8	21.3	9	16.8	13	17.1	10
HSG0620311113	16	15.9	11	7.9	18	16.6	11	17.9	12	23.4	7	20.3	9	17.0	11
RCAT PINEHURST	17	14.6	15	11.1	14	19.1	7	17.1	14	20.3	12	17.1	12	16.5	13
S12A5	18	15.7	12	14.8	7	20.9	5	23.6	5	23.5	6	21.2	7	20.0	7
$LSD_{0.05}$		5.2		5.2		4.3		4.4		5.9		6.8		2.1	

INK11: Inkerman 2011
ROS11: Ross Farm 2011
SAB11: Ste. Anne-de-Bellevue 2011
INK12 : Inkerman 2012
KEN12 : Kent Ag 2012
SAB12: Ste. Anne-de-Bellevue 2012
Ave.: Average

The genotype with the greatest δ-tocopherol concentration across sites and in all six environments was DH530 (entry #4) (Table 13). This genotype was also had very stable concentration across sites (Figure 17). One interesting characteristic for this particular tocopherol was that all genotypes except for entry #3 (DH4202) and #16 (HSG0620311113) had highly stable concentrations across sites. This suggests that this particular trait is largely inherited with little impact of the environment on concentrations.

Finally, γ-tocopherol concentration was also the greatest in DH530 (entry #4) which ranked in the top four genotypes in all six individual environments (Table 14). However, it was also one of the least stable genotype (Figure 18). The genotype with the greatest stability was entry #6 (DH715L), which ranked fourth overall in terms of γ-tocopherol concentration across environments. Results thus demonstrate that it is possible to identify genotypes that will have both high and stable concentrations of specific tocopherols across environments. Such characteristic is essential for the nutraceutical industry as it ensure the supply of consistent raw material for product development, which is a key constraint for the industry, especially when labeling is involved.

Table 12. Total tocopherol concentrations (µg/g DM) in 18 genotypes grown in six environments in Ontario and Quebec

Table 2. Total tocopherol concentrations (ug/g DM) in 18 genotypes grown in 6 environments in Ontario and Quebec.

Genotype	#	INK11	Rank	ROS11	Rank	SAB11	Rank	INK12	Rank	KEN12	Rank	SAB12	Rank	Avg.	Rank
DH4052	1	408.8	8	414.7	8	371.5	13	391.4	8	421.7	9	387.7	12	399.3	8
DH4100CN	2	430.9	4	426.7	4	440.3	2	442.2	3	485.5	1	426.9	2	442.1	2
DH4202	3	381.3	13	370.5	15	403.2	8	362.1	14	374.3	17	390.1	11	380.3	14
DH530	4	461.3	1	467.5	1	478.4	1	448.7	1	484.1	2	456.5	1	466.1	1
DH6177	5	398.2	11	417.6	7	404.7	7	373.8	11	425.3	8	409.1	6	404.8	7
DH715L	6	430.4	5	429.1	3	424.3	4	446.5	2	470.9	3	420.6	4	437.0	3
HS0506a11117	7	401.1	10	388.7	12	375.1	12	358.2	15	402.9	10	368.5	16	382.4	12
HS05111117	8	418.1	7	424.1	6	439.7	3	406.5	6	438.4	6	426.6	3	425.6	5
HS0511H24	9	442.3	3	426.0	5	357.3	17	414.5	5	443.4	5	400.9	7	414.1	6
HS0511H32	10	419.9	6	389.2	11	411.0	5	374.7	10	396.7	12	398.9	8	398.4	9
HS052411157	11	370.6	14	380.7	13	388.8	10	358.0	16	401.7	11	387.0	13	381.1	13
HS053211119	12	395.0	12	380.0	14	400.4	9	367.1	12	385.9	14	390.2	10	386.4	11
HSG0616611111	13	366.1	16	370.3	16	366.7	15	379.4	9	379.7	16	347.5	17	368.3	17
HSG0616711111	14	443.4	2	453.9	2	409.6	6	427.3	4	455.6	4	415.7	5	434.2	4
HSG0616711113	15	405.4	9	389.9	10	367.0	14	394.3	7	393.4	13	375.4	14	387.6	10
HSG0620311113	16	368.7	15	358.1	17	361.2	16	365.5	13	428.7	7	395.4	9	379.6	15
RCAT PINEHURST	17	326.8	18	326.6	18	340.5	18	326.7	18	371.9	18	338.7	18	338.5	18
S12A5	18	349.1	17	400.5	9	376.5	11	341.6	17	383.9	15	375.3	15	371.1	16
$LSD_{0.05}$		32.42		34.66		39.66		29.51		33.41		37.45		17.97	

Table 13. Delta-tocopherol concentrations (µg/g DM) in 18 genotypes grown in six environments in Ontario and Quebec

Table 3. Δ-tocopherol concentrations (ug/g DM) in 18 genotypes grown in 6 environments in Ontario and Quebec.

Genotype	#	INK11	Rank	ROS11	Rank	SAB11	Rank	INK12	Rank	KEN12	Rank	SAB12	Rank	Avg.	Rank
DH4052	1	137.1	9	140.3	9	135.2	8	124.7	8	132.5	9	121.0	11	131.8	8
DH4100CN	2	173.0	2	171.9	2	156.7	4	162.1	3	168.4	2	156.6	2	164.8	2
DH4202	3	117.7	15	126.5	15	137.9	7	107.0	17	109.0	16	116.7	13	119.1	16
DH530	4	178.4	1	191.3	1	169.1	1	165.5	2	173.6	1	160.7	1	173.1	1
DH6177	5	150.5	6	152.1	7	142.7	5	135.1	6	138.9	8	135.9	5	142.5	6
DH715L	6	167.8	3	166.7	4	158.7	2	166.2	1	164.8	3	145.6	3	161.6	3
HS0506a11117	7	136.5	10	133.1	13	127.3	13	114.9	13	128.1	10	113.2	15	125.5	12
HS05111117	8	153.1	4	153.9	5	157.5	3	136.6	5	146.2	5	143.4	4	148.4	4
HS0511H24	9	145.7	7	150.5	8	128.9	12	130.7	7	139.0	7	125.2	9	136.7	7
HS0511H32	10	137.3	8	137.4	11	133.6	10	118.9	11	123.5	13	121.0	12	128.6	10
HS052411157	11	122.2	14	127.7	14	125.9	14	109.7	16	122.3	14	113.5	14	120.2	14
HS053211119	12	134.1	11	134.9	12	133.8	9	123.7	9	126.6	11	123.6	10	129.5	9
HSG0616611111	13	112.1	17	117.2	16	114.6	16	115.0	12	104.5	18	101.8	17	110.9	17
HSG0616711111	14	152.7	5	168.7	3	139.7	6	140.8	4	150.0	4	129.5	7	146.9	5
HSG0616711113	15	122.4	13	138.2	10	114.9	15	118.9	10	120.7	15	111.5	16	121.1	13
HSG0620311113	16	112.7	16	113.7	17	112.8	17	111.1	15	139.3	6	130.1	6	120.0	15
RCAT PINEHURST	17	108.2	18	110.3	18	108.5	18	97.9	18	106.1	17	98.1	18	104.8	18
S12A5	18	125.1	12	152.7	6	130.9	11	111.3	14	124.6	12	125.3	8	128.3	11
$LSD_{0.05}$															

Table 14. Gamma-tocopherol concentrations (µg/g DM) in 18 genotypes grown in six environments in Ontario and Quebec

Table 4. Γ-tocopherol concentrations (ug/g DM) in 18 genotypes grown in 6 environments in Ontario and Quebec.

Genotype	#	INK11	Rank	ROG11	Rank	GAB11	Rank	INK12	Rank	KEN12	Rank	GAB12	Rank	Avg.	Rank
DH4052	1	252.3	6	257.4	3	217.8	16	243.1	9	264.2	8	245.1	12	246.6	8
DH410GCN	2	248.1	9	246.7	8	269.1	2	265.1	2	301.7	1	260.5	4	265.2	3
DH4202	3	247.3	10	232.0	16	248.9	7	235.4	11	246.7	14	255.1	6	244.2	10
DH530	4	266.9	3	263.4	2	287.5	1	263.5	4	290.5	2	272.9	1	274.1	1
DH6177	5	231.3	15	253.6	6	244.0	9	220.8	16	259.3	9	253.9	7	243.8	11
DH715L	6	249.5	8	252.1	7	251.5	6	264.9	3	285.8	4	259.0	5	260.5	4
HG0506a11117	7	249.9	7	243.0	9	233.9	13	226.1	14	255.2	11	241.0	15	241.5	13
HG05111117	8	246.6	11	253.9	4	263.2	3	244.4	8	265.6	7	262.7	3	256.1	5
HG0511H24	9	267.9	2	253.6	5	212.0	18	252.9	6	266.2	5	245.2	11	249.6	6
HG0511H32	10	256.7	5	234.1	14	254.1	5	227.4	12	244.5	16	249.9	9	244.4	9
HG0524111157	11	230.1	16	237.9	12	240.4	10	226.7	13	256.3	10	252.1	8	240.6	15
HG0532111119	12	239.4	13	228.9	17	245.5	8	220.9	15	240.5	17	242.9	14	236.3	16
HGG0616611111	13	239.2	14	241.3	10	234.0	12	248.4	7	254.3	12	229.5	16	241.1	14
HGG0616711111	14	277.2	1	275.5	1	256.7	4	269.7	1	286.0	3	269.7	2	272.5	2
HGG0616711113	15	266.5	4	239.1	11	238.0	11	254.1	5	251.4	13	247.1	10	249.4	7
HGG0620311113	16	240.1	12	236.5	13	231.8	14	236.5	10	266.0	6	245.0	13	242.6	12
RCAT PINEHUR0T	17	204.0	18	205.3	18	213.0	17	211.6	17	245.5	15	223.5	18	217.1	18
012A5	18	208.3	17	233.0	15	224.7	15	206.7	18	235.7	18	228.9	17	222.9	17
$LSD_{0.05}$															

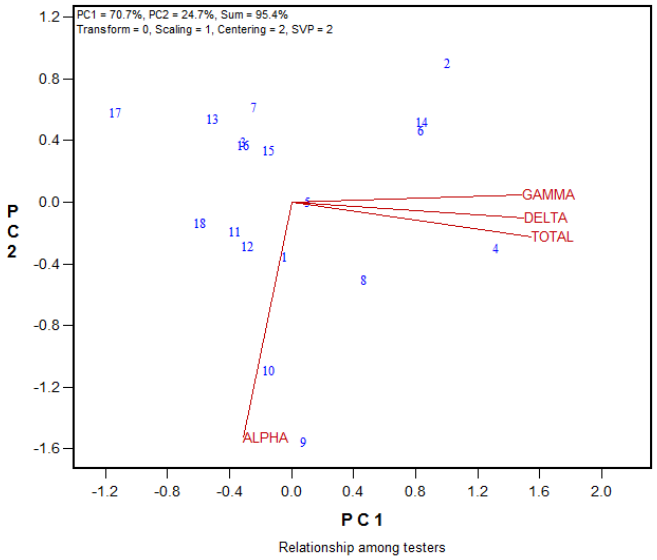


Figure 14. Genotype by trait bi-plot across six environments.

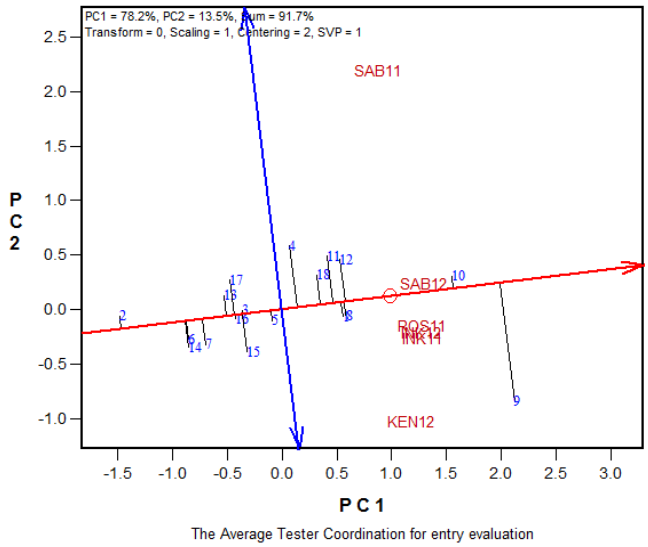


Figure 15. Mean vs. Stability bi-plot for α-tocopherol.

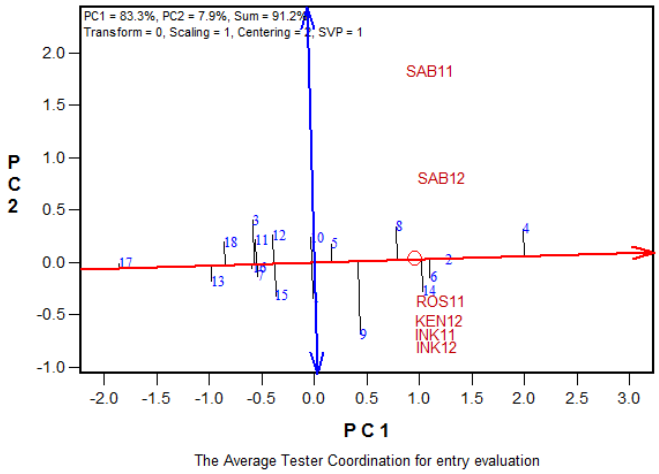


Figure 16. Mean vs. Stability bi-plot for total tocopherol.

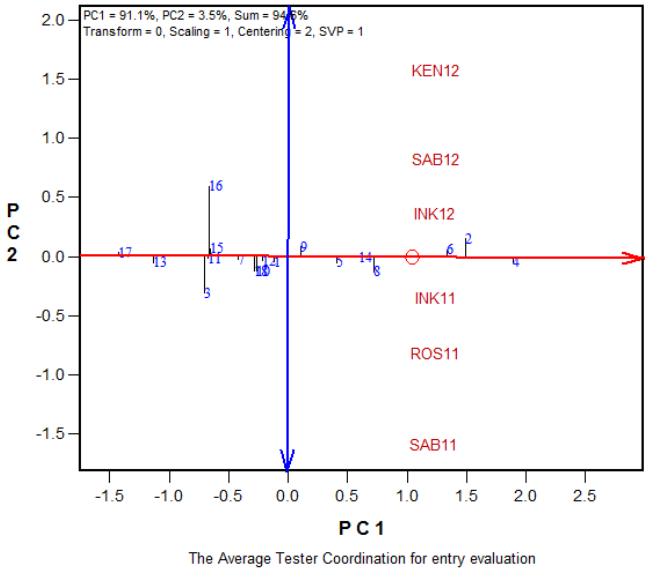


Figure 17. Mean vs. Stability bi-plot for δ-tocopherol.

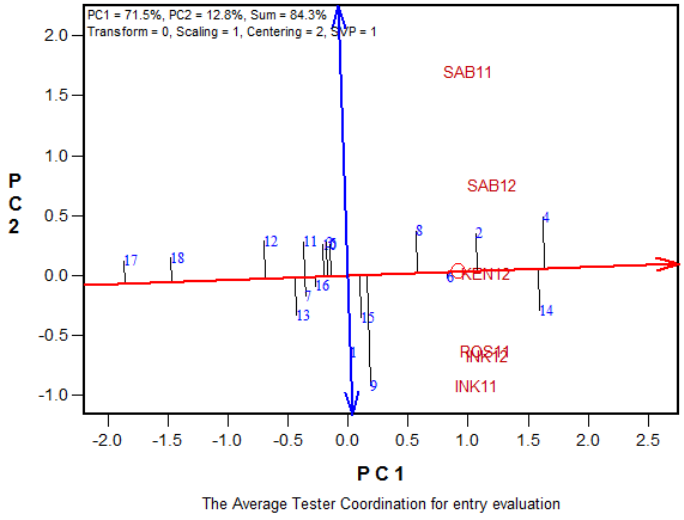


Figure 18. Mean vs. Stability bi-plot for γ-tocopherol.

Objective 2. Relationship between tocopherols concentrations, value-added traits and other important seed characteristics.

The second objective was to identify possible relationships between tocopherol concentrations, other value-added traits and other important agronomic characteristics. To do so, samples were selected in 2010, 2011 and 2012 from the multi-location trials of Sevita International and a total of 156 samples were analyzed for a maximum of 30 variables. Correlations between these variables revealed several significant correlations between individual and total tocopherols and other traits of importance. The significant correlations are presented in Table 15. There were a total of six positive and seven negative correlations for α-tocopherol, seven positive and six negative for δ-tocopherol, seven positive and ten negative for γ-tocopherol, and six positive and eight negative for total tocopherol.

The correlations with the highest Pearson correlation coefficients for α-tocopherol were those with days to maturity and days to flowering (i.e. r=-0.54 and -0.50, respectively). Similar strong correlations with days to maturity were also observed for γ-tocopherol and total tocopherol (i.e. r=-0.72 and -0.52, respectively). These correlations suggest that highest concentrations of all tocopherols, except for δ-tocopherol, should be observed in earlier maturing soybeans. A negative correlation was also observed between these three tocopherols and seed yield (i.e. r ranging between -0.40 and -0.64) (Figures 19, 20 and 21). These results contrast with the earlier observations of Seguin et al. (2009), that reported a lack of correlation between tocopherols and seed yield. Although some significant correlations were observed between tocopherols and some other nutraceutical traits such as isoflavones and lutein, none had a Pearson correlation coefficient greater than ±0.40. In accordance with Seguin et al. (2009) no correlation between tocopherols and other important seed traits such as protein and oil content has been observed.

Results from the study suggest that it should be possible to select concurrently for several nutraceutical traits, with highest tocopherol concentrations expected in earlier maturing genotypes. The negative correlation between α-, γ- and total tocopherol and seed yield is of concern and might be an impediment in selecting both high yielding and high tocopherol material.

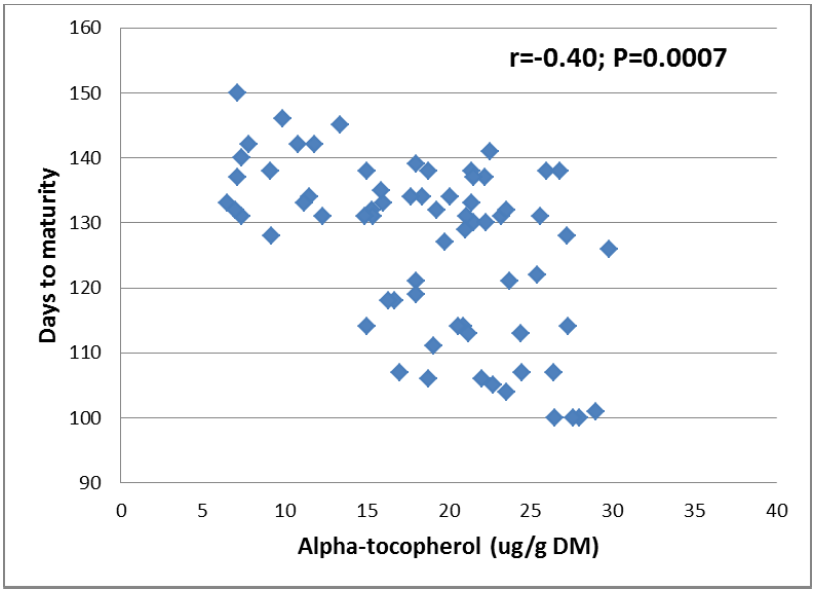


Figure 19. Correlation between α-tocopherol concentration and days to maturity among cultivars and advanced breeding lines of Sevita International.

Table 15. Significant correlations (P < 0.05) between tocopherol concentration and other important agronomic and seed quality characteristics

Table 5. Significant correlations (P < 0.05) between tocopherol concentrations and other important agronomic and seed quality characteristics.

	Positive Correlations	Negative Correlations
α-tocopherol	Linoleic acid C18:2 (r=0.44; P=0.005) Soyasapogenol B (r=0.30; P=0.009) Total soyasapogenol (r=0.28; P=0.01) γ-tocopherol (r=0.26; P=0.0008) Lutein (r=0.23; P=0.004) Total tocopherol (r=0.21; P=0.01)	**Days to maturity (r=-0.54; P<0.0001)** **Days to flowering (r=-0.50; P<0.0001)** Seed yield (r=-0.40; P=0.0007) δ -tocopherol (r=-0.27; P=0.0005) Daidzein (r=-0.24; P=0.0002) Total isoflavones (r=-0.21; P=0.008) Genistein (r=-0.18; P=0.02)
δ-tocopherol	**Total tocopherol (r=0.64; P<0.0001)** Fatty acid C18:3 (r=0.33; P=0.04) Days to maturity (r=0.31; P<0.01) Sucrose (r=0.29; P=0.007) γ-tocopherol (r=0.24; P<0.003) Genistein (r=0.21; P=0.007) Total isoflavones (r=0.20; P=0.01)	α-tocopherol (r=-0.28; P=0.0005) Lutein (r=-0.20; P=0.02) Soyasapogenol A (r=-0.23; P=0.04) Soyasapogenol B (r=-0.23; P=0.04) Total soyasapogenol (r=0.26; P=0.02) Plant emergence (r=-0.36; P=0.008)
γ-tocopherol	**Total tocopherol (r=0.89; P<0.0001)** Soyasapogenol B (r=0.40; P=0.0003) Total soyasapogenol (r=0.39; P=0.0005) Lutein (r=0.28; P=0.0004) Glycitein (r=0.27; P=0.0008) α-tocopherol (r=0.26; P=0.0008) δ -tocopherol (r=0.24; P=0.002)	**Days to maturity (r=-0.72; P<0.0001)** **Seed yield (r=-0.64; P<0.0001)** **Lodging (r=-0.57; P<0.0001)** **Seed weight (r=-0.54; P<0.0001)** **Days to flowering (r=-0.51; P<0.0001)** Plant height (r=-0.45; P<0.0001) Plant emergence (r=-0.45; P=0.0005) White mould (r=-0.40; P=0.0007) Daidzein (r=-0.24; P=0.002) Total isoflavones (r=-0.18; P=0.01)
Total tocopherol	**γ-tocopherol (r=0.89; P<0.0001)** **δ -tocopherol (r=0.64; P<0.0001)** Glycitein (r=0.27; P=0.0005) Soyasapogenol B (r=0.25; P=0.03) Total soyasapogenol (r=0.23; P=0.05) α-tocopherol (r=0.21; P=0.01)	**Days to maturity (r=-0.52; P<0.0001)** **Lodging (r=-0.51; P<0.0001)** Seed yield (r=-0.47; P<0.0001) Plant emergence (r=-0.46; P=0.0004) Days to flowering (r=-0.39; P=0.001) Seed weight (r=-0.37; P=0.002) White mould (r=-0.37; P=0.0002) Plant height (r=-0.33; P=0.006)

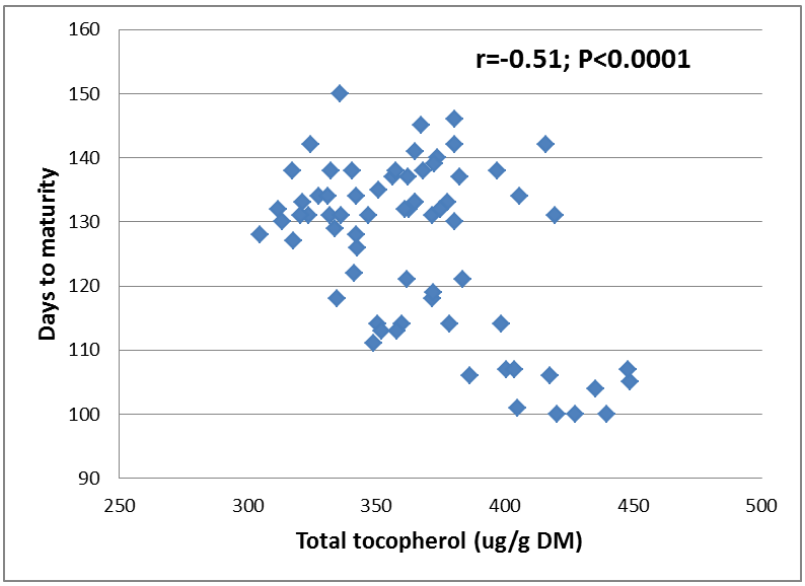


Figure 20. Correlation between total tocopherol concentration and days to maturity among cultivars and advanced breeding lines of Sevita International.

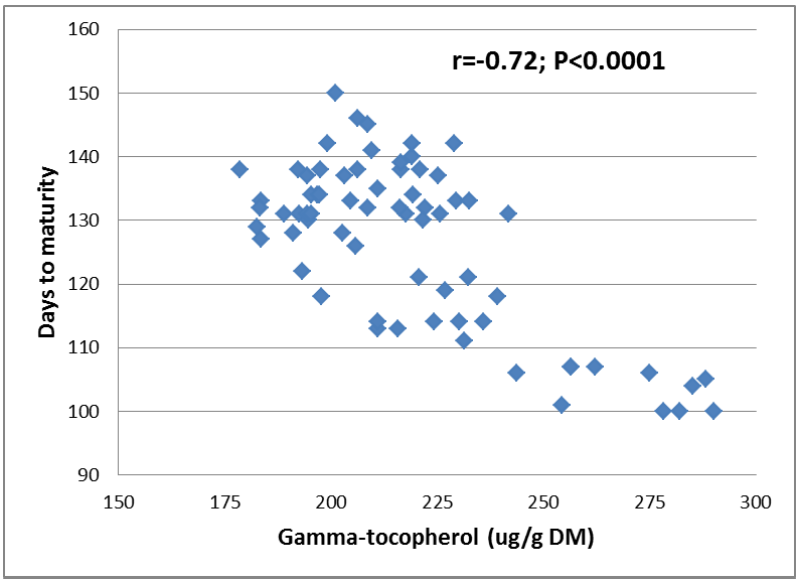


Figure 21. Correlation between γ-tocopherol concentration and days to maturity among cultivars and advanced breeding lines of Sevita International.

Conclusions and Next Steps

The project was successful as it achieved all its objectives. With the first objective, cultivars and advanced breeding lines were identified with high and stable tocopherol concentrations;

1. HS0511H32,
2. DH530,
3. DH715L,
4. DH410SCN

for α-, δ-, γ- and total tocopherol, respectively. This information helps Sevita International in making decisions regarding material to be released and should lead to the release of material that will be marketed.

As a result of the project, one variety from Sevita International's germplasm has been flagged as a high tocopherol and will continue into F8 filial stage yield evaluation trials at Sevita International in 2013. This variety has the potential to become registered in 2014 and may be available for export as soon as the fall of 2017.

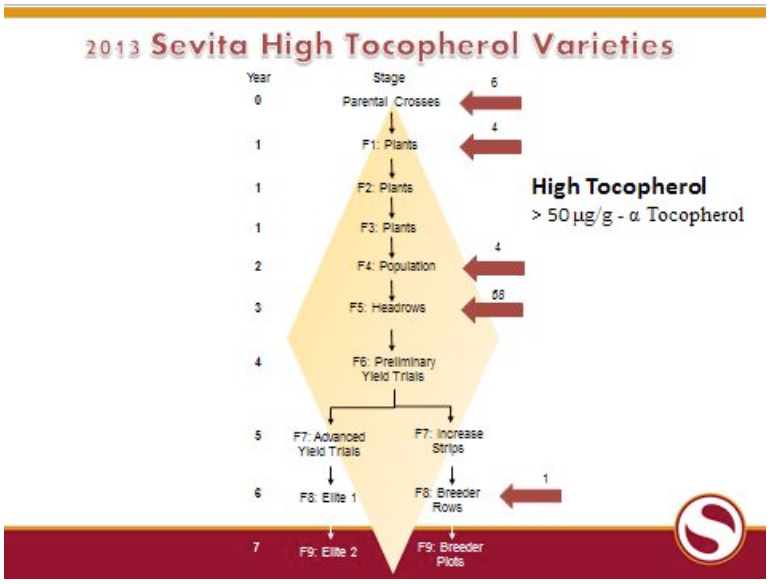


Figure 22: High tocopherol varieties in Sevita International's germplasm.

With the second objective, useful information was provided regarding correlations between tocopherols and other important traits in food-grade soybeans. The data will help the breeding program of Sevita International to determine if multiple traits could be maximized in a single genotype, and will help them in prioritizing traits which will be selected for.

Finally, the project also allowed for the development of a productive collaboration between the industry (ECODA, Sevita International) and McGill University. This fruitful collaboration led to the submission of another project for funding through the AgriScience program. The intention was to use a similar approach to that used in objective 1 to identify high and stable genotypes for lutein concentration and also for some biomarkers to be linked to consumer preference in food-grade soybean. The interaction, collaboration and contribution of McGill University, Sevita International and ECODA is effectively strengthening the innovation of the Canadian food-grade soybean sector and ultimately will make it more competitive on the world-market.

Objective 2 - This information has helped Sevita International in identifying, cataloguing and making decisions regarding materials to be released. The following catalogues have been confirmed at filial stage F7 as an outcome of this sub-activity:

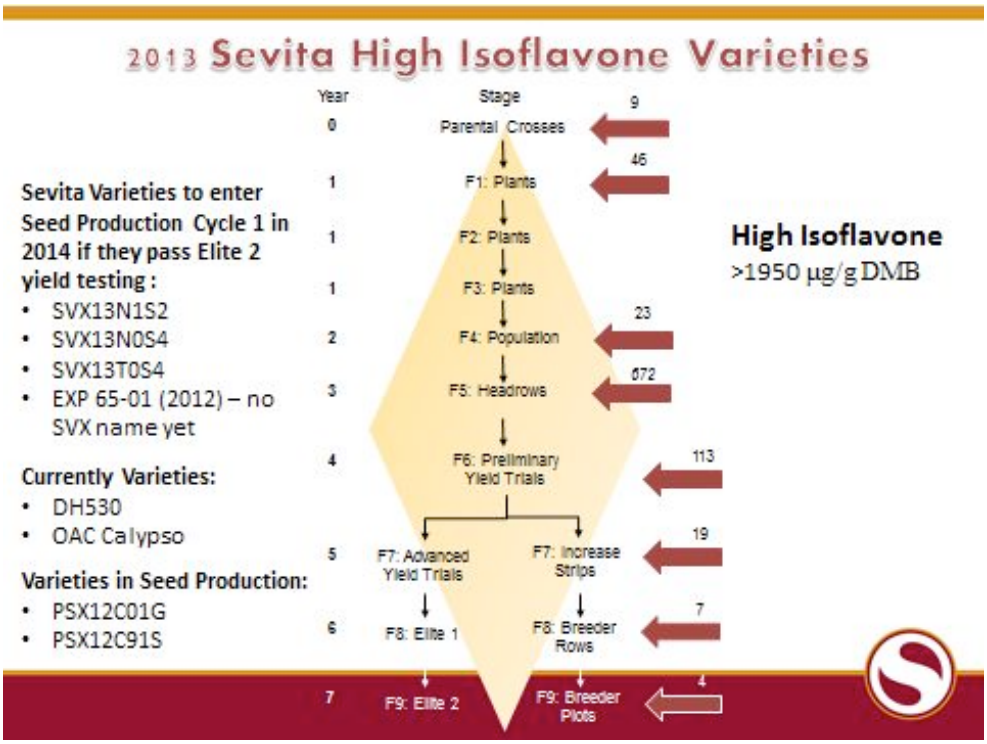


Figure 23: High isoflavone varieties in Sevita International's germplasm.

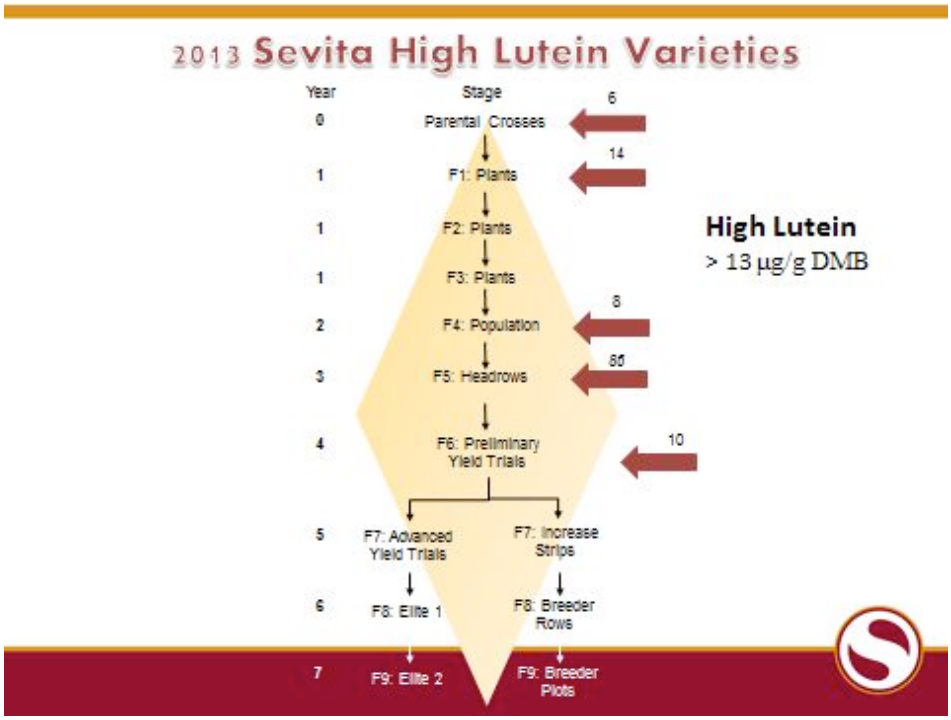


Figure 24: High lutein varieties in Sevita International's germplasm.

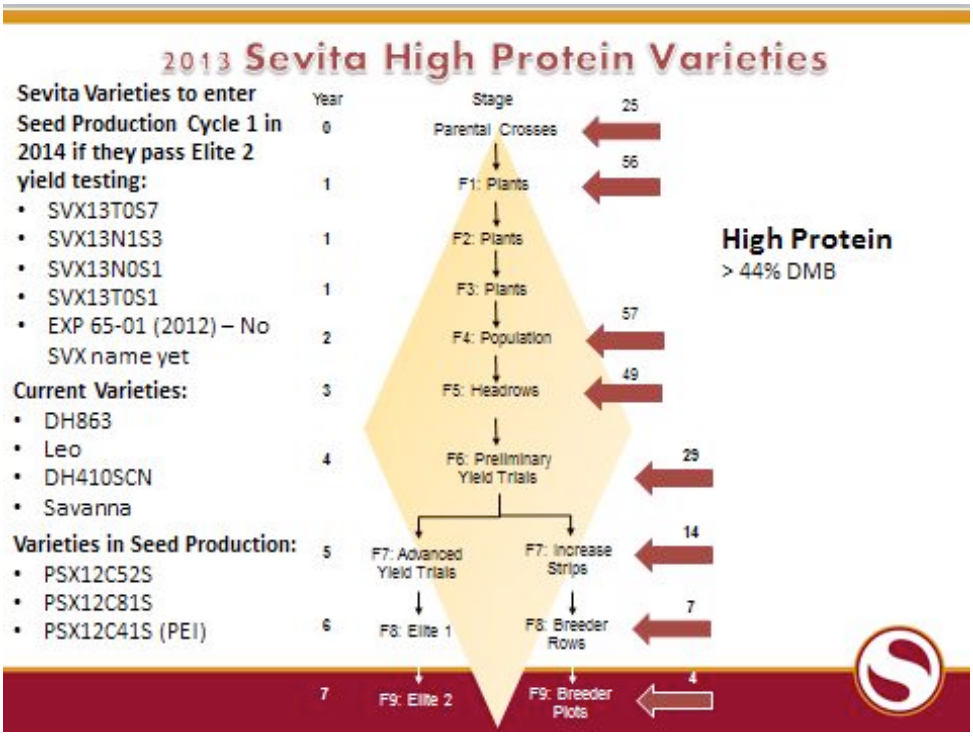


Figure 25: High protein varieties in Sevita International's germplasm.

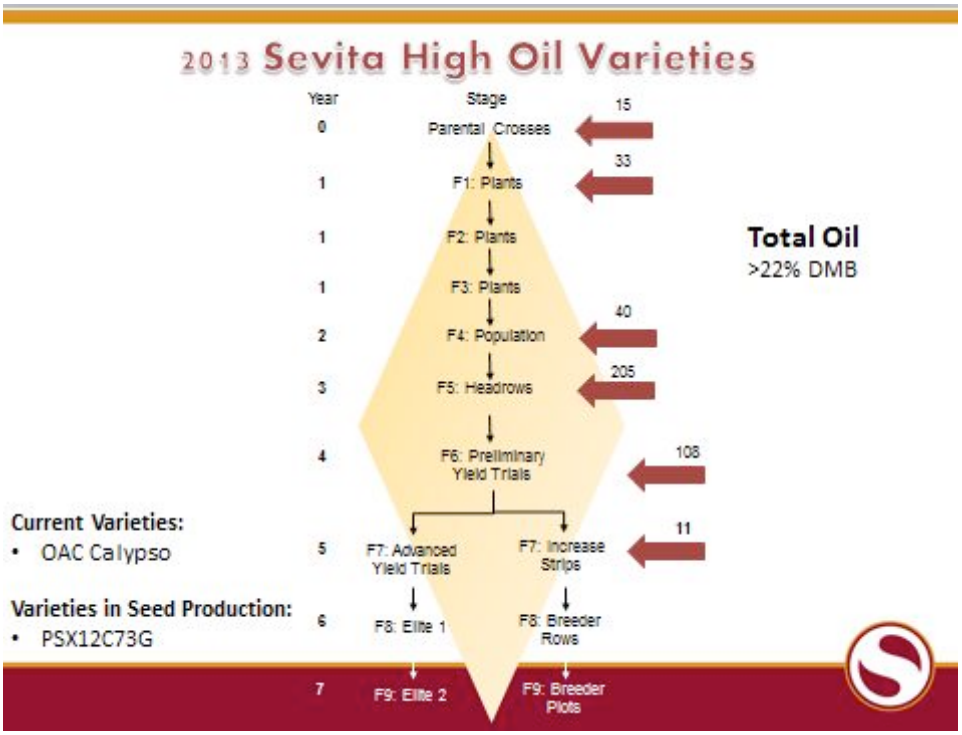


Figure 26: High oil varieties in Sevita International's germplasm.

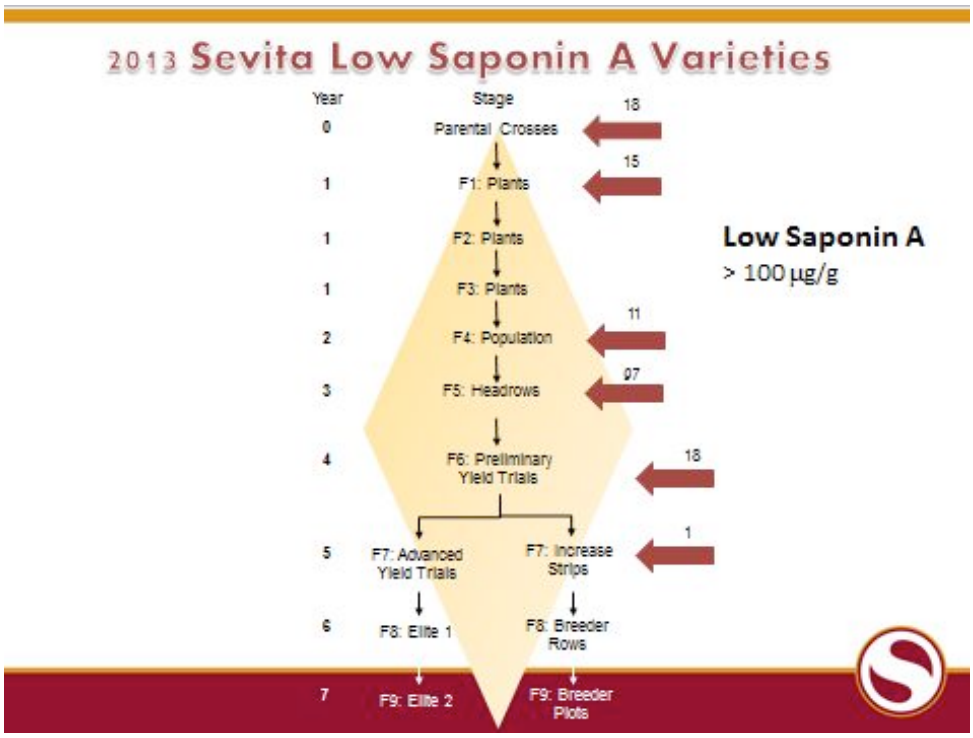


Figure 27: Low Saponin A varieties in Sevita International's germplasm.

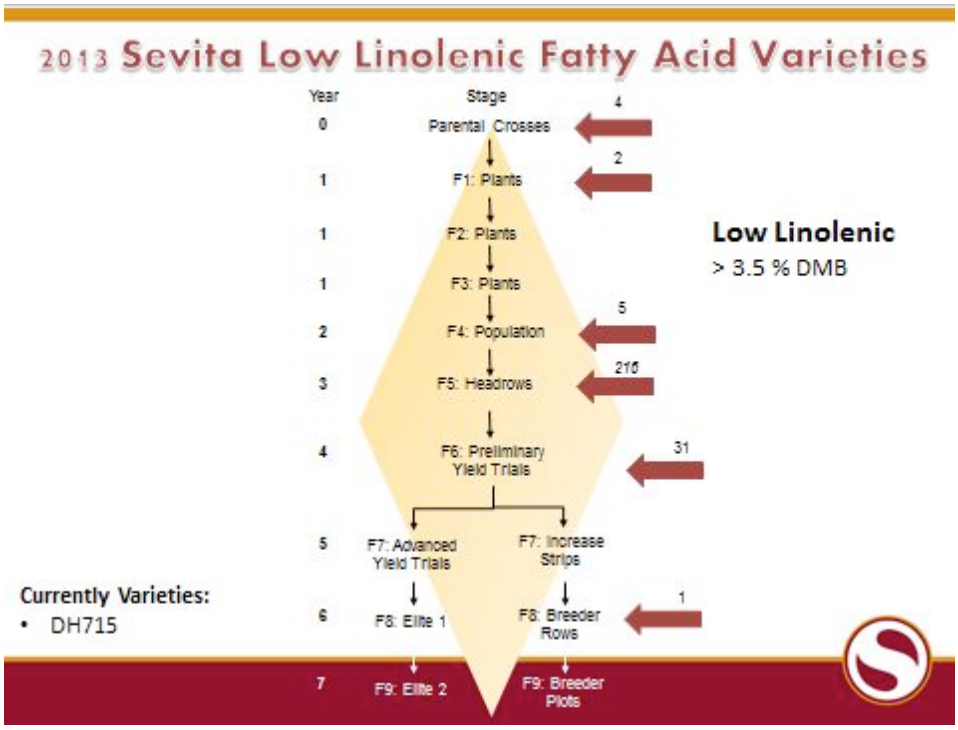


Figure 28: Low linolenic varieties in Sevita International's germplasm.

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1.4 Food grade soybeans with improved quality for the Japanese and European Union

Objective

The objective of this sub-activity is to develop food-type soybean cultivars which meet specific quality criteria of the Japanese and European Union buyers.

Performance Indicators

Tofu/soymilk cultivars were identified in the course of evaluating various populations, which can be commercialized by Sevita International.

Methodology

For this project, several populations were identified which had been developed at Harrow from crosses between adapted food quality soy breeding lines or cultivars and lines with the quality traits of improved protein subunit composition (i.e. null for α' and A4 subunits), high sucrose and lipoxygenase null. Lines from these populations, which are designated as ECODA lines, are progressing through the Harrow breeding program and, should any of them reach the cultivar release stage, ECODA/Sevita International would be offered the right of first refusal to commercialize them. Based on 2011 results, two of the high sucrose lines, SE06-0337LML-3 and SE06-0337LLM-1, had very good yields, both in Harrow, Ontario and Woodslee, Ontario and overall, had high levels of total free sugars, although the protein content and seed size could be improved upon. These lines were tested in the Advance Yield trials in 2012 (Tables 16 & 17).

The testing results of SE06-0337LML-1 and its checks are summarized in Table 16. SE06-0337LML-1 had slightly higher sucrose content than the checks, OAC Thamesville and S26-F9, but significantly higher than the check Tourco. It yielded slightly lower than OAC Thamesville, but higher than S26-F9 and Tourco. It had significantly lower

protein content than Tourco, but similar to the other two checks, OAC Thamesville and S26-F9. The free sugar content of SE06-0337LML-1 was significantly higher than S26-F9 and Tourco, but slightly lower than OAC Thamesville. The seed size of SE06-0337LML-1 was significantly smaller than Tourco, but similar to OAC Thamesville and S26-F9. The total hydrolysable carbohydrates of SE06-0337LML-1 were significantly higher than S26-F9 and Tourco. SE06-0337LML-1 matured three days later than Tourco and nine days later that OAC Thamesville and S26-F9. It had similar oil content to the checks. Although the plant height of SE06-0337LML-1 was 15 to 20 cm higher than the checks, its resistance to lodging is similar to the checks.

Table 16. Performance of Experimental line SE06-0337LLM-1 and check cultivars OAC Thamesville, S26-F9 and Tourco in Southwest Ontario, 2012 Advanced Yield Late (AL) AAFC trials[z].

Cultivar	Seed								Plant	
	Yield	Maturity	Weight	Prot[y]	Oil[y]	Sugar[y]	Suc[y]	Carb[yw]	Height	Lodging
	(t/ha)	(d)	(g/100sd)	%	%	%	%	%	(cm)	(1-5)[x]
SE06-0337LLM-1	3.135	136	21.0c	40.4c	21.7	12.2bc	7.0c	18.9bc	103	1.5
OAC Thamesville	3.141	127	20.6	41.2	21.0	12.4	6.9	18.7	83	1.0
S26-F9	2.943	127	19.2	40.9	21.9	11.8	6.8	18.1	86	1.0
Tourco	2,881	133	25.8	43.4	21.2	11.4	6.4	17.7	88	1.3
No. Sites	3	3	3	3	3	3	3	3	2	2

[z]Performance based on three trials conducted at Harrow, Holiday Beach and Woodslee in 2012 (AL12).
[y]Whole dry matter basis, by near infrared spectroscopy.
[x]1=no lodging to 5= complete lodging
[w]Carb, Total hydrolysable carbohydrates
[abc]Significantly different ($p<0.05$) from OAC Thamesville, S26-F9 and Tourco, respectively.

The testing results of SE06-0337LML-3 are summarized in Table 17. SE06-0337LML-3 yielded lower than OAC Thamesville and S26-F9, but higher than OAC Kent. It had a significantly larger seed size than S26-F9 and significantly higher total hydrolysable carbohydrates content than OAC Kent. The protein content, oil content, free sugar content and sucrose content of SE06-0337LML-3 were similar to the checks. SE06-0337LML-3 matured significantly later than the checks. Although its plant height was significantly higher than OAC Kent, it had fair lodging resistance.

Of the low lipoxygenase lines evaluated in this program, SQ05-0026M-3lx-3 has the best potential for commercialization as it combines excellent seed size and protein level with relatively early maturity and decent lodging resistance, a weakness in many low lipoxygenase lines. This line was tested in the 2012 Miscellaneous Breeder Yield Trial (BYM12) and the testing results are summarized in Table 18.

SQ05-0026M-3lx-3 is lipoxygenase 1, 2 and 3 null. It yielded less than the check OAC Thamesville. It had a significantly larger seed size and significantly higher protein contents than OAC Thamesville. The oil, free sugar and total hydrolysable carbohydrates contents of SQ05-0026M-3lx-3 were significantly lower that the check OAC Thamesville. It matured two days later than OAC Thamesville and had 7cm higher plant height than the check, but similar resistance to lodging as OAC Thamesville. Currently, Sevita International is evaluating this line for its soymilk quality in the Japanese market.

In summary, although progress has been made towards the development of food quality soybean for specific traits, there are still some bottlenecks that need to be overcome. The yield of these lines is still low; the sucrose content is not high enough.

Table 17. Performance of Experimental line, SE06-0337LML-3 and check cultivars OAC Thamesville, S26-F9 and OAC Kent in Southwest Ontario, 2012 Advanced Yield Middle-Late (AML) AAFC trials[z].

Cultivar	Seed								Plant	
	Yield	Maturity	Weight	Prot[y]	Oil[y]	Sugar[y]	Suc[y]	Carb[yw]	Height	Lodging
	(t/ha)	(d)	(g/100sd)	%	%	%	%	%	(cm)	(1-5)[x]
SE06-0337LML-3	2,957	138_{abc}	22.2_{b}	41.3	21.4	11.9	6.7	18.7_{c}	105_{c}	2.25
OAC Thamesville	3.164	124	20.3	41.7	20.8	12.3	7.0	18.5	87	1.0
S26-F9	3.219	125	19.9	41.9	21.4	11.9	6.6	17.9	89	1.0
OAC Kent	2,738	124	20.7	41.2	22.2	11.8	6.3	17.4	84	1.0
No. Sites	3	3	3	3	3	3	3	3	2	2

[z]Performance based on three trials conducted at Harrow, Holiday Beach, and Woodslee in 2012 (AL12).
[y]Whole dry matter basis, by near infrared spectroscopy.
[x]1=no lodging to 5= complete lodging
[w]Carb, Total hydrolysable carbohydrates
[abc]Significantly different (p<0.05) from OAC Thamesville, S26-F9, and OAC Kent, respectively.

Table 18. Performance of Experimental line, SQ05-0026M-3lx-3 and check cultivar OAC Thamesville in Southwest Ontario, 2012 Breeder Yield Mis. (BYM12) AAFC trials[z].

Cultivar	Seed								Plant	
	Yield	Maturity	Weight	Prot[y]	Oil[y]	Sugar[y]	Suc[y]	Carb[yw]	Height	Lodging
	(t/ha)	(d)	(g/100sd)	%	%	%	%	%	(cm)	(1-5)[x]
SQ05-0026M-3lx-3	3.496	131	29.2_{a}	46.7_{a}	19.3_{a}	11.5_{a}	6.23_{a}	16.9_{a}	101_{a}	1.7
OAC Thamesville	3.857	129	21.1	42.4	20.7	12.1	6.67	18.3	94	1.3
No. Sites	6	5	6	6	6	6	6	6	5	5

[z]Performance based on three trials conducted at Harrow, Holiday Beach, and Woodslee in 2012 (AL12).
[y]Whole dry matter basis, by near infrared spectroscopy.
[x]1=no lodging to 5= complete lodging
[w]Carb, Total hydrolysable carbohydrates
[a]Significantly different (p<0.05) from OAC Thamesville.

Conclusions and Next Steps

Tofu cultivars identified for commercialization in the course of the several population evaluations have been identified. These cultivars will undergo further testing to determine if they will be commercialized by Sevita International.

Sevita International also focused on the development of varieties with the quality traits of improved protein subunit composition (i.e. null for α' and A4 subunits), high sucrose content and lipoxygenase null.

Over the course of the project the below varieties were developed. These varieties will continue through the variety development process and may result in a commercialized variety being commercially available as soon as the fall of 2016.

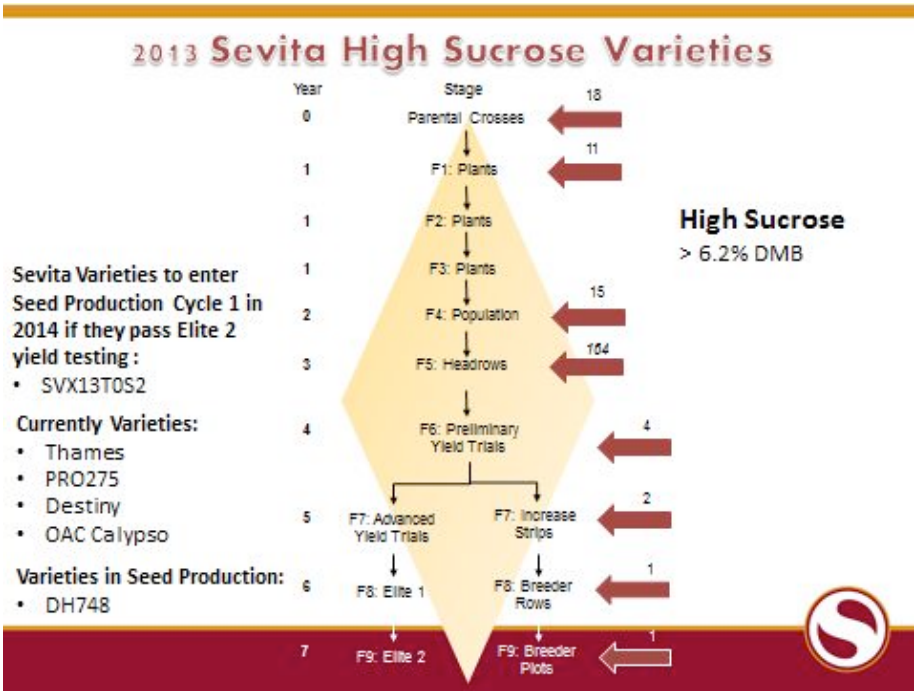

Figure 29: High Sucrose varieties in Sevita International's germplasm.

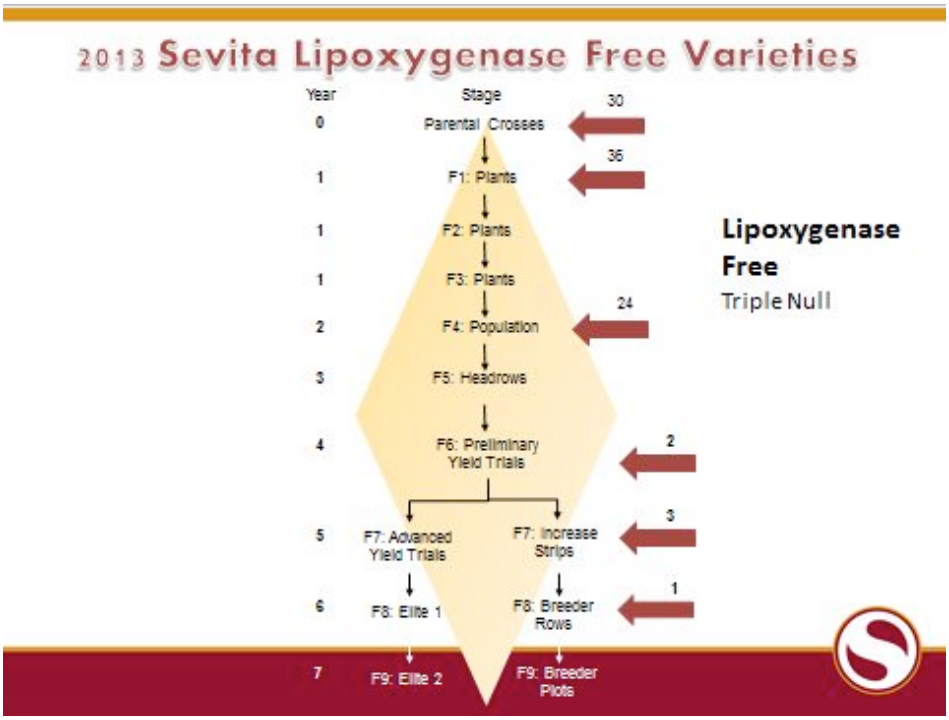

Figure 30: Lipoxygenase Free varieties in Sevita International's germplasm.

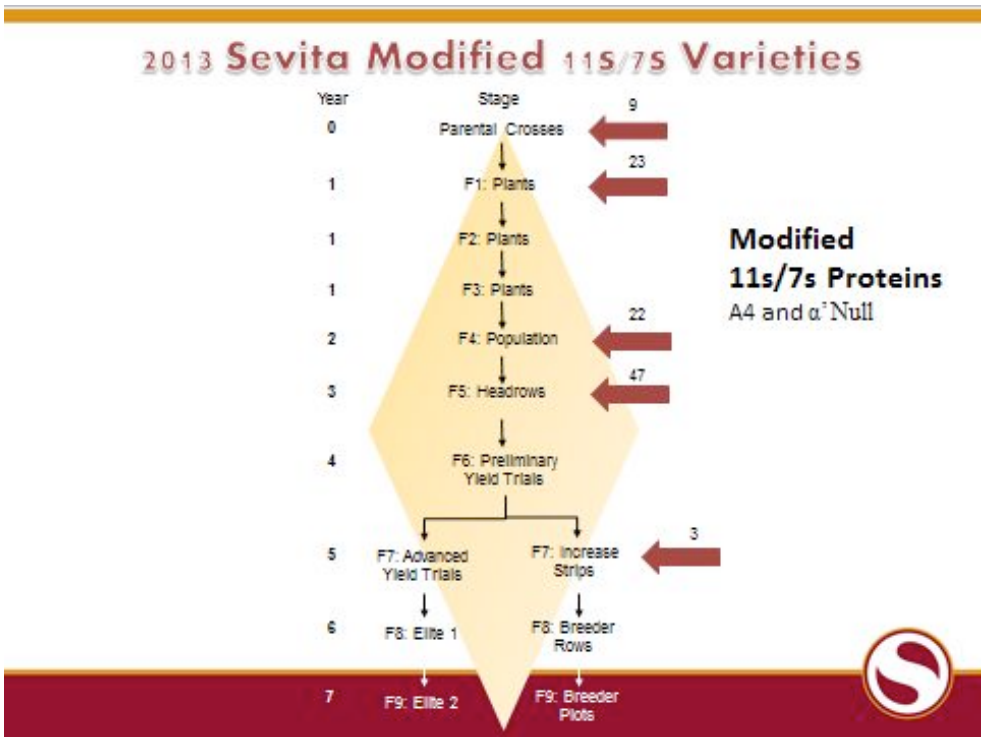

Figure 31: Modified 11S/7S varieties in Sevita International's germplasm.

B (I). Funded Collaborators (Co-PI, AAFC, other federal scientists)

- Include the name of scientist / organization.

AAFC Scientists
Elroy Cober, Malcolm Morrison, Ottawa
Richard Martin, Charlottetown
Vaino Poysa and Kangfu Yu, Harrow

McGill University
Philippe Seguin

Sevita International
Jagdish Kumar, David Hendrick

University of Guelph
Istvan Rajcan

AAFC RESEARCH BRANCH
Research Project Final Report

B (II). Acknowledgement of non-funded collaborators (who provide support, e.g. access to other laboratory or other facilities and equipment input / advice / guidance / assistance, etc).

- For research supported by targeted funding programs (e.g. DIAP, Clusters, etc.) please list any collaborators who are receiving Contribution Vote 10 funds (e.g., university and industry collaborators). In addition, please list separately the participants who support your project but are not receiving any funding through the program.
- Include name of scientist / organization.

N/A

C. Variance Report (if applicable, describe how the work differs from the proposed research)

- Include changes to objectives and project work plan / budget, changes to the team, other constraints.

At AAFC Harrow, Vaino Poysa retired and was replaced by Kangfu Yu, however, the work continued as planned.

D. Impact Assessment (if applicable, describe how the variance factors above will impact project continuation)

- Include changes to the objectives, changes to the project work plan / budget, changes to performance (i.e. meeting targets).

A number of soybean cultivars developed and breeding lines tested and developed has had a great impact on achieving the objectives of developing food grade, value-added, high yielding and nutraceutical soybean cultivars and germplasm for Canadian farmers using Canadian and Chinese sources. A number of cultivars were developed and identified which are uniquely adapted to production in Eastern Canada.

E. Achievements (include only those related to this project)

- Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs.

Refer to ECODA DIAP Final Performance Report.

F. Lessons learned (self-evaluation of project)

The project has had an impact on the soybean variety and germplasm development in Canada using domestic, exotic (e.g. Chinese) and mutant soybean sources to develop traits such as low linolenic, zero lipoxygenase, high tocopherol (vitamin E), high linoleic and high yielding as well as increased protein content cultivars.
In general terms, the project was successful as it achieved all its objectives. It allowed for the development of a productive collaboration between the industry (ECODA, Sevita International) and McGill University. The overall DIAP project also allowed for inter-institutional collaboration with other organizations like AAFC. These interactions and collaborations contribute in strengthening the innovation of the Canadian food-grade soybean sector.

AAFC RESEARCH BRANCH
Research Project Final Report

Jim McCullagh	May 29 , 2013	
PI Name	Date	Signature

Note: After completion and signature, this report must be provided to the appropriate Science Director for assessment. A PDF copy of this report will be sent to Science Operations by the Science Director's office along with the project assessment.