Agricultural Innovation Program
Research Project Final Report
Contribution Agreement - Vote 10 Funding

Project Title:	Development of a Designer Soybean Testing Methodology Activity 4: Variety Development and Fingerprinting / Genotype by Environment Interaction
Start Date (yyyy-mm-dd):	2012-04-01
Expected End Date (yyyy-mm-dd):	2013-03-31
Actual End Date (yyyy-mm-dd):	2013-03-31
Principal Investigator (PI):	Sevita International – Jim McCullagh

Short Executive Summary of report:

ECODA's major soybean industry partner, Sevita International, exports a wide range of soybean varieties to Japan for use by Japanese processors to make soymilk and tofu. Each Japanese customer has a different formulation, process and style of product and therefore different soybean varieties work better for some customers than others.

Previous activities of this project identified compounds that had a significant influence on the taste of tofu and soymilk. The current activity studied the expression of these biomarkers across a number of different growing locations and also tested a number of parents being used by the Sevita International breeding program.

This activity addresses two objectives:
1) determine which phytochemicals are common between parents and progeny; and
2) evaluate the interaction of these phytochemicals with the environment.

A series of head to head trials were conducted to assess genotype by environment interactions with respect to the biomarkers that have been identified in previous activities in this project. All parents in the Sevita International breeding program were grown during the 2012 growing season in Inkerman, Ontario, to identify parents that have high and low expression of the identified biomarkers.

After screening, the variety DH863 was found to have a reduced expression of compounds that contribute to a 'beany' taste in tofu. The variety DH618 was shown to have high levels of a compound that enhances sweetness in soymilk. Both DH863 and DH618 were used in bi-parental crosses in the summer and winter of 2012. Progeny of these parents were also advanced in the program and will be ready for agronomic assessment in the 2013 growing season.

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.

Introduction

Crop failure can be a medium to high risk due to factors out of control to farmers. In order for a company like Sevita International to decrease their risk of production, they need to consider offering varieties which are stable

and can be grown, with consistent results, in different geographic areas. If this can be achieved, the risk of production decreases and the potential to meet the needs of customers increases.

As export customers expect a certain degree of consistency across any particular variety, it is important to understand how a variety is affected within each of its potential growing areas. Thus, studying genotype by environment (GxE) interactions is of particular interest as they contribute to the understanding of how various compounds are affected by changes in growing conditions.

In plant breeding, there must be a significant underlying genetic component in the expression of a compound for plant breeders to reliably select for a trait in question. For a breeding program to actively breed for a specific compound of interest, plant breeders must also have an understanding of what genetic variation exists in the program. Finally, plant breeders must also have an understanding of how that variation is transferred into the progeny of a cross between two parents. This variation can then be selected for or against in the breeding program as the progeny is developed.

To accurately assess the stability of a given trait, testing must be conducted across a number of different growing conditions over a number of growing seasons using a number of different varieties. Based on a successful pilot, this project was the first year of what will be a multi-year experiment attempting to determine the stability of biomarker expression in NON- genetically modified (GM) soybean varieties.

To investigate the genotype by environment (GxE) relationship, Sevita International chose to test its current commercially available line-up and upcoming experimental varieties against other varieties of soybeans common in the industry. Appendix 1 lists the varieties involved in the head to head trial as well as the locations at which each variety was tested.

A sample of seed from each variety from each location was sent to the University of Ottawa for analysis using a state of the art ultra-high performance liquid chromatography with time-of-flight mass spectrometry (UPLC MS QTOF) analytical facility.

To better understand the relationship between the parents used in Sevita International's soybean breeding program and the progeny that they produce, samples of their germplasm were sent for analysis at the University of Ottawa. The University screened potential breeding parents for the biomarkers identified in activity 3 of this project: high sweetness for soymilk and low beaniness for tofu. Potential breeding parents displaying the desirable biomarkers, listed above, were flagged. Progeny produced from varieties flagged for desirable biomarkers were then tracked through the breeding program and will undergo further evaluation at the F5 (headrow) stage of development.

Objective

- Variety development and fingerprinting: determine the effects of plant breeding on the presence of the essential phytochemical compounds.
- Variety development and fingerprinting: test the parents of the known acceptable and unacceptable varieties and create crosses with parents known to have the identified biomarker compounds.
- Genotype by environment interaction: perform 'head to head' multi-location trials and evaluate the interaction between the growing environment and the presence of the essential phytochemical compounds.

Deliverables

- A summary of findings.

Method – Head to Head Trials

Sevita International's varieties as well as other common soybean varieties were identified (Appendix 1) and grown in multiple locations across Canada, the United States and Europe. Sevita International cleaned, counted,

packaged and shipped seed to each collaborator with information and instructions as follows:

Variety Identification

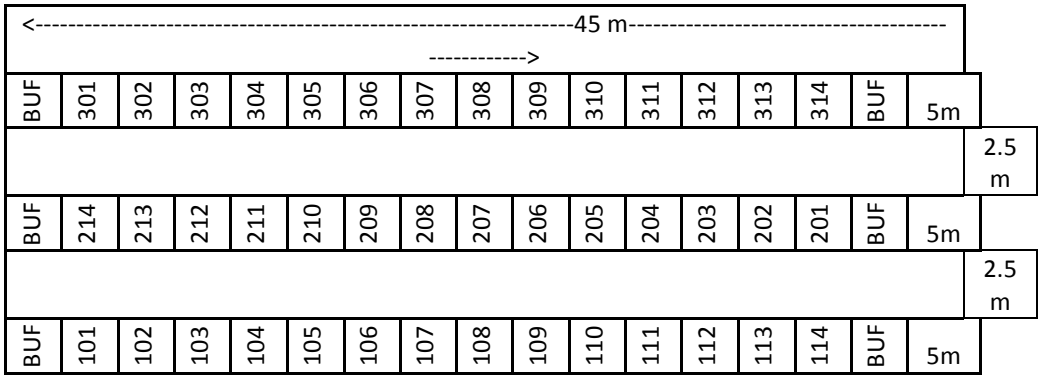
- Number of entries/experiments included in the shipment (not including borders/buffer/guards).
- Information on each packet included: location, experiment number, plot number and entry number.

Planting Instructions

- Each tier was to be planted in sequential order (Plot # 101 to 114 / 201 to 214 / 301 to 314).
- Sevita International was to be advised if barcode field labels and stakes were required.
- If asked to insert local checks (controls), appropriate seed quantities were to be inserted in packets labelled CHECK1 or CHECK2 or etc. and advise which varieties were used as checks.

Below an example is presented of how Sevita International would plant this trial.

Figure 1: Example of a 14 entry, three repetitions yield trial. (four rows per plot with 15"spacing)



Data to be Supplied to Sevita International

The supplied Data Field Document contains the parameters of information to capture for each plot.

1. Plot yield in grams and in bushels per acre (bu/ac).
2. Moisture % per plot.
3. Grams per 100 seeds.
4. Emerged plants per plot (only note if emergence is low).
5. Plant height in centimeters at R6 stage (R6 stage soybean plant is full seed, i.e. the pod contains green seed that fills the pod cavity at one of the four uppermost nodes of the main stem).
6. Days to maturity (this is when 95% of pods are brown, day 1 =planted).
7. Pubescence (brown, tawny or grey).
8. Lodging (1 to 5, 5 being flat).
9. Disease (indicate what disease and score (1=0-10%, 2=10-25%, 3=25-50%, 4=50-75%, 5=75-100%).
10. Insect damage (1=0-10%, 2=10-25%, 3=25-50%, 4=50-75%, 5=75-100%).
11. Overall visual plot score 1-10 (10 is the best). To be taken at the same time as days to maturity.
12. Comments (off-types, soil issues, herbicide damage, water damage etc.) .
13. Indicate plot dimensions.
14. Date of planting and harvesting.
15. Soybean seeds (500g) were sent to Sevita International for food and seed analysis, and food data via near-infrared spectroscopy (N.I.R.), where applicable.

Harvest Instructions

- Every harvest bag was to be labelled during harvest - preferably with the experiment number, location, plot number and entry number.
- Once the data was entered in the excel data field document, a soft copy was to be sent to Sevita International. Also, if plot stakes were supplied, the stakes were to be returned.
- Once bags had been analyzed, Sevita International was to be contacted for pick up or shipment instructions.

Sevita International also grew head to head trials in their home nursery location of Inkerman, Ontario, as well as in their offsite location sites in Ontario (Kinburn, Packenham, Chrysler, Williamstown, Williamsburg and Kemptville) and in Quebec (Shawville). The trials were planted with 30 entries (i.e. varieties) per trial and three plots per entry. Plots were spread throughout the trial in a randomized layout. After harvest, a sample from each of the three plots was combined into a representative sample for each variety. These representative samples were then sent to the University of Ottawa for analysis on the ultra HPLC MS QTOF.

Method – Head to Head Trial Evaluation

80% methanol extraction

1) Grind 2g of soybean seeds in a Wiley mill (Arthur H. Thomas Co.) through a 1mm mesh (size 20).
2) Weight accurately 1.0g of ground material and extract with 10ml of 80% methanol (bulk, Fisher Scienfitic) by shaking at 200rpm for 60 min.
3) Centrifuge at 4000rpm for 10 min. at 25°C (Eppendorf, 5810R) and decant the supernatant.
4) Re-extract residuals with 5ml of methanol by shaking at 200rpm for 60 min.
5) Repeat step 3 and then combine the supernatant.
6) Evaporated the supernatant to dryness by blowing air in a fume hood.

Hexane wash and preparation for UPLC-Q-TOF analysis

1) Re-solubilize dried extract in 1ml of 80% methanol (LCMS grade, Fisher Scientific) by sonication for 5 min. in a glass vial.
2) Add 1ml hexane (optima grade, Fisher Scientific) to the vial, hand shake the vial three to five times, wait for 15min. for separation.
3) Transfer the hexane layer to another glass vial.
4) Repeat step 2 to 3 two times.
5) Adjust the final volume of the 80% methanol layer to 5ml by 80% methanol.
6) Filter 1ml of extract into a HPLC vial by PTFE syringe filters (0.22mm, Whatman).
7) Prepared sample are store at –20°C until analysis.

UPLC-QTOF analysis

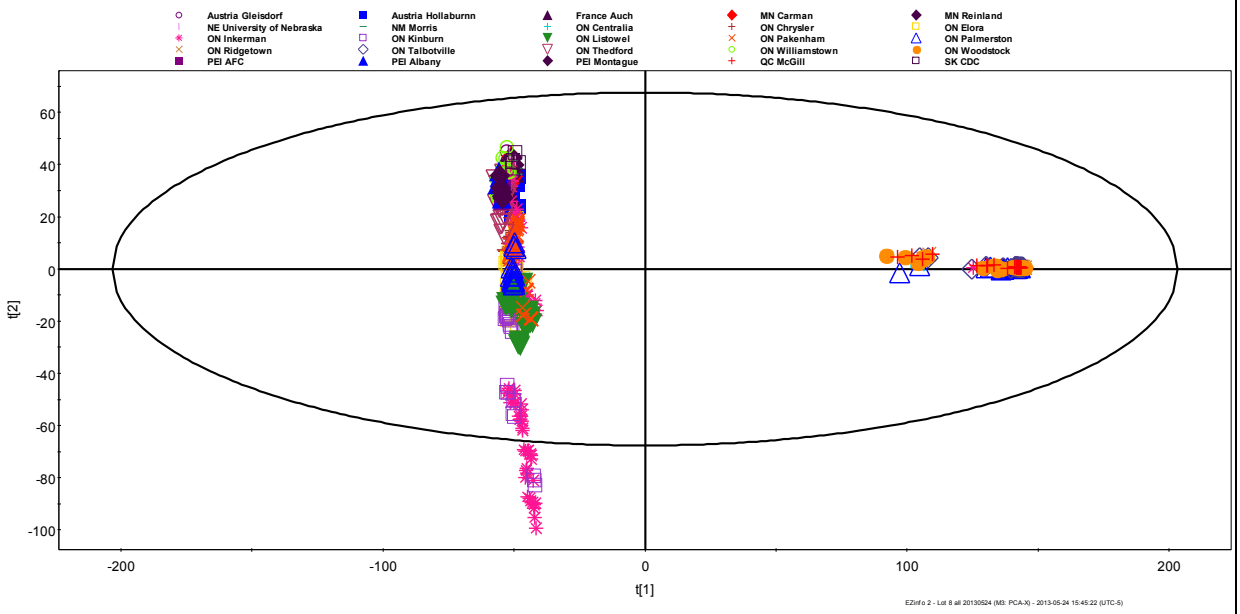
Optimized UPLC conditions: Acquity CSH C18 1.7um 2.1 x 100mm column (part #186005297; lot #0113320401) connected with a VanGuard Pre-column 2.1 x 5mm. Mobile phase, A, water+0.1% formic acid, B-acetonitrile+0.1% formic acid (Fisher Optima LC-MS). Flow rate 0.5ml/min. Column temperature, 50°C, sample temperature 25°C. Mobile phase composition, 0-1min 5% A isocratic, 1-6min linear gradient 5-50% B, 6-8min 50-95%B, 8.01-10min 5% A isocratic (total run time: 10min). Optimized sample injection conditions: 1ul injection, weak wash 600ul (10% acetonitrile+90% water), strong wash 200ul (90% acetonitrile+10% water). Optimized QTOF analysis conditions: MassLynx software, MSe ESI+ mode, lock mass Leucine Enkephalin ^{12}C 556.2615, source temperature 120°C, desolvation temperature 400°C, cone gas (N2) flow 50l/hr, desolvation gas (N2) flow 1195l/hr. MSe conditions, mass range 100-1500Da, F1 CE, 6V, F2 CER 10-30V, Cone voltage 20V, Scan time 1sec. Calibration, 50-1000Da sodium formate. Optimized statistical analysis conditions: Principal component analysis (PCA) and discriminate analysis (OPLS-DA) were performed by MarkerLynx. Pareto scaling was performed after grouping all the samples.

Results and Discussion – Head to Head Trials

Of the 791 total samples included in this study, the University of Ottawa has analyzed 320 samples selected by Sevita International. The samples were collected from 31 locations. This includes 12 locations in Ontario, three locations in Manitoba, three locations in Prince Edward Island, one location in Quebec, three locations in Saskatchewan, two locations in Austria, two locations in France and five locations in the United States of America.

The 320 sample dataset including all varieties was subjected to PCA analysis (Figure 2) and the environment effects on the metabolome are clearly visible. Results show clear separation of the varieties grown in Woodstock, Talbotville, Ridgetown, Palmestron, McGill and Centralia, grouping on the positive side of the first axis and away from other varieties. Locations in Europe cluster on the positive end of the second axis while Inkerman and Kinburn cluster towards the second axis.

Figure 2: PCA analysis of metabolome 320 accessions grown in different environments

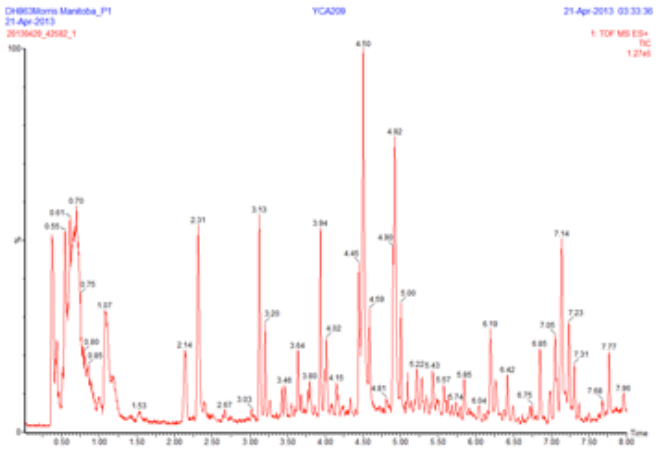


Differences in individual chromatograms are illustrated in Figure 3, which show different fingerprints for the genotype DH863 for Morris MB and Pakenham ON.
In Figure 4, even greater effects are seen in the fingerprint for DH4173 grown in Austria or McGill. The dataset for these two genotypes at 25 locations is available in Appendix 2. Considering that there are 300 identified compounds in each genotype and 25 locations for each genotype, this becomes a very large data set. This data set was critical in determining how the environment affects taste and odour compounds in the product.

These samples have been analyzed on the ultra HPLC MS QTOF for the full metabolome. Results show clear separation of the varieties grown in Woodstock, Talbotville, Ridgetown, Palmestron, McGill and Centralia from all other varieties. Varieties grown in Inkerman seem to divide into two groups: one closely related to Kinburn and the other group related to the rest of the varieties. European varieties did not show separations from the ones grown in USA or Canada.

Figure 3: Differing metabolomic fingerprints for DH863 grown in Pakenham, ON and Morris, MB illustrating environmental effects

Morris Manitoba



Pakenham

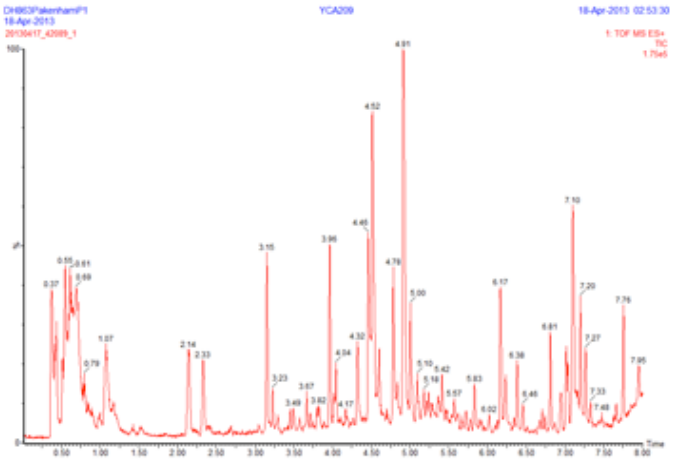
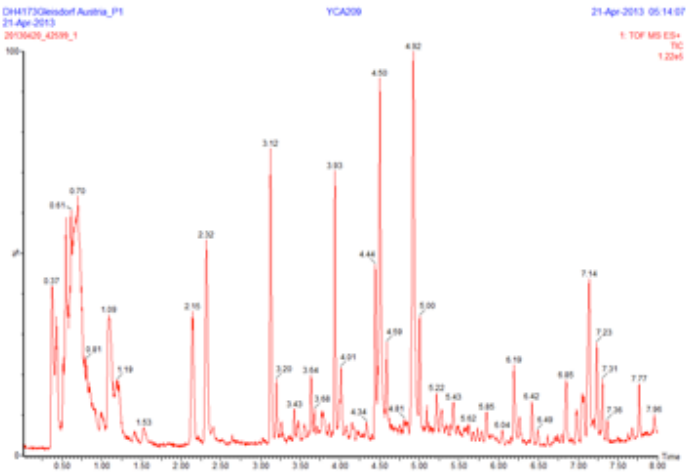
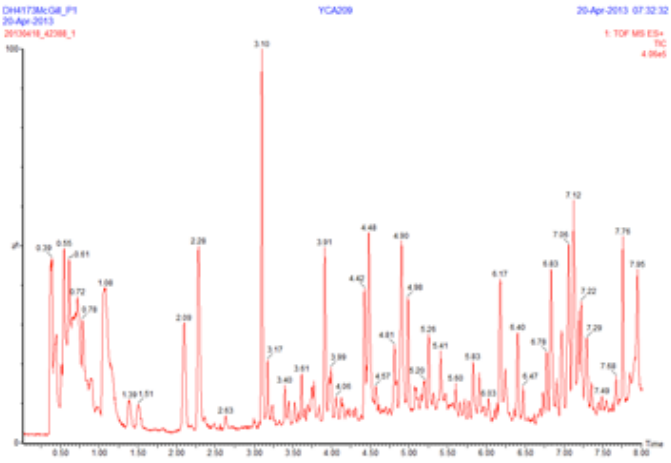


Figure 4: Differing metabolomic fingerprints for DH4173 grown in Austria and McGill illustrating environmental effects

Gleisdorf Austria



McGill

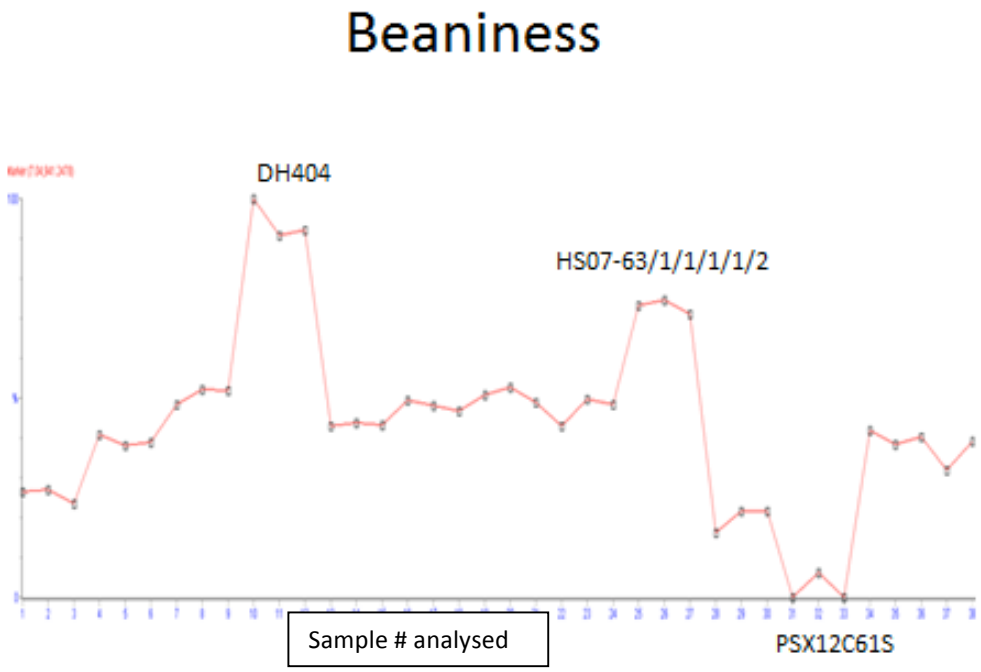


Results and Discussion – Parent Evaluation, Crosses, Progeny Advancement and Selection

The biomarkers were identified for key characteristics, and breeding lines were analysed for the presence of each biomarker. High biomarker genotypes that are identified can then be used as parents in breeding for desirable cultivars. Genotypes were grown at a common location to minimize environmental effects associated with metabolite expression.

Figure 5 illustrates a biomarker (rt= 3.95min, m/z= 254.929 da) for low beaniness in preferred tofu varieties, which has been mapped across genotypes. Clearly, several genotypes (DH404 replicated in samples 11, 12 and 13 and HS07-63/1/1/1/2 replicated in samples 25, 26 and 27 on the x axis) have exceptional levels of the biomarker and are available to develop elite or "designer" varieties. Selection of a low biomarker variety (PSX12C61S replicated in samples 31, 32, 33) for crosses to the high biomarker genotypes can also provide parents for crossing and variety development.

Figure 5: Occurrence of the bean flavour biomarker (rt= 3.95min, m/z= 254.929) across genotypes.



Similarly, a biomarker for sweetness (rt= 7.04 min, m/z = 941.248) was mapped across genotypes in Figure 6. The variety PSX12C52S appears to have a high concentration of a biomarker that contributes to sweetness in soymilk while the variety DH401 appears to have lower levels of this biomarker.

Figure 6: Occurrence of the soymilk sweetness biomarker (rt= 3.95min, m/z= 254.929) across genotypes.

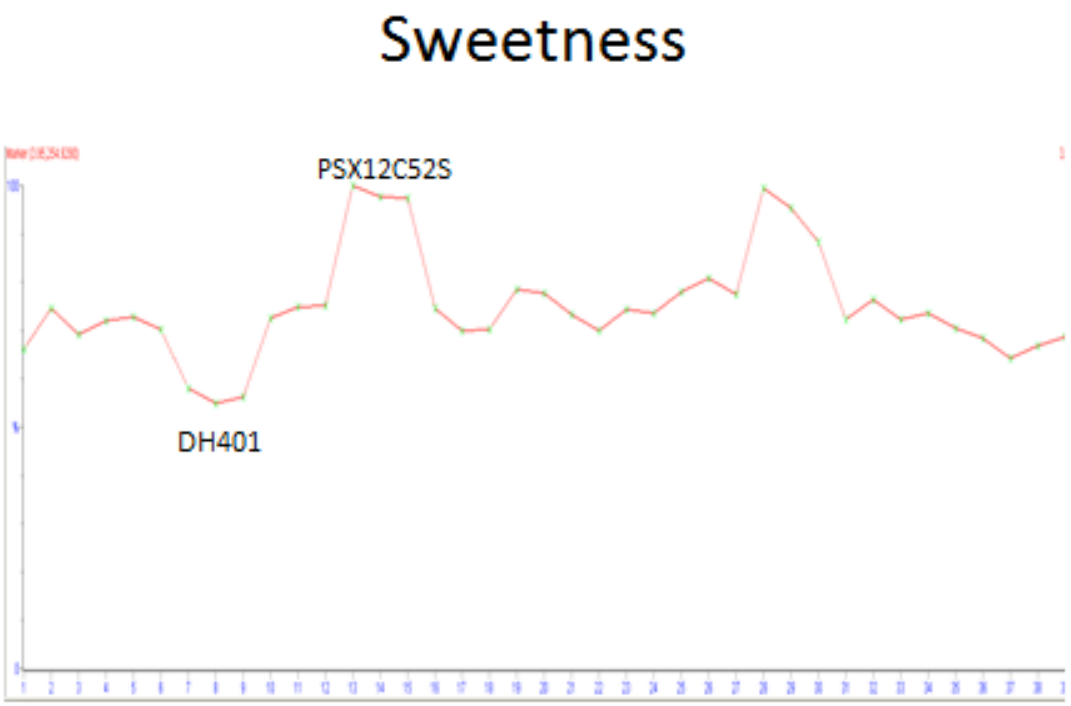


Table 1: Parents used for bi-parental crosses in 2012.

Sample	Parent
1	PSX12C41SInkerman
4	DH863Inkerman
7	DH401-3Inkerman
10	DH404Inkerman
13	PSX12C52SInkerman
16	DH420Inkerman
19	DH618Inkerman
22	HS06-31/1/1/1/1/7cInkerman
25	HS07-63/1/1/1/1/20cInkerman
28	SavannaInkerman
31	PSX12C61SInkerman
34	HS06-28/1/1a/1/1/8aInkerman
37	S03-W4Inkerman

Parents for bi-parental cross pollinations, as presented in Table 1, were selected using data generated from metabolomic screening. Crosses were made with DH863 for reduced beaniness (Table 2, Table 4) and DH618 (Table 3) for increased sweetness in tofu and soymilk, respectively. These parents were often crossed with varieties that exhibit superior agronomic performance to ensure the progeny has sufficient yield potential. Cross pollinations were conducted in accordance with standard operating practices at Sevita International.

Table 2: Crosses conducted in summer 2012 for 'low beaniness'

Cross #	Seeds Produced	Pods Produced	Crossing attempts	Female parent	Male parent
12-392	4	2	7	DH863	DH618
12-393	3	1	6	DH863	OAC MADOC
12-394	6	4	8	DH863	DH880
12-395	12	5	10	DH863	DH402
12-399	6	2	5	DH4202	DH863
12-401	1	1	3	BEIJIANG296	DH863
12-419	4	2	5	DH863	STARGAZER
12-420	1	1	8	DH863	HEINONG 48
12-433	4	2	3	DH402	DH863
12-437	2	1	4	HUINANPINGDINGXIANG	DH863
12-444	6	2	5	DH863	DH4173
12-451	1	1	3	DH863	IAR 2001 BSR
12-456	8	4	8	DH863	AC MERSEA
12-457	11	6	6	DH863	SC2307
12-458	3	2	4	DH863	IVORY
12-513	5	2	4	DH863	OAC WALLACE
12-530	3	1	5	DH863	IAR 2001 BSR
12-531	5	2	6	PSX12C91S	DH863
12-552	16	7	9	DH863	IA2053LF
12-553	2	1	6	DH863	HSV03-01/23/1
12-554	1	1	3	DH863	OAC KENT
12-555	2	1	5	DH863	SC2307
12-556	6	3	6	DH863	RCAT 1003
12-557	6	2	2	DH863	KANGXIAN98-98
12-559	6	3	5	DH863	P09-153/3LFLS
12-560	2	1	4	DH863	0AR-76237 SCN
12-561	3	1	5	DH863	IA2040LF
12-562	7	3	7	DH863	P09-31/4LFLS
12-563	2	1	3	DH863	IVORY
12-584	7	3	7	DH863	RCAT 1002
12-614	3	1	3	ISIDOR	DH863
12-618	3	1	3	DH863	OAC HURON
12-633	5	3	14	DH863	0AR-73232 SCN
12-634	5	2	14	DH863	209F.HP SCN
12-635	3	1	6	DH863	DH777
12-657	9	4	8	DH863	BAUMANN BLACK
12-661	14	5	7	DH863	SUZUMARU
12-662	9	5	12	DH863	Katrina x Yu ci Huang
12-680	5	2	9	DH863	CX1834-1-6
12-683	3	1	9	OAC WALLACE	DH863
12-705	5	2	6	DH863	NATSU DAIZU
12-767	5	2	7	DH863	TANISHI
12-783	14	5	5	DH863	OAC PERTH
12-805	1	1	5	DH863	Micron
12-816	4	2	3	DH863	KK21-B12
12-822	1	1	4	DH863	KENJIANGDOU 43CB3
12-823	4	2	6	ZAOFENG 918	DH863
12-845	10	5	5	DH863	ENREI
12-872	3	2	3	DH863	E11

Table 3: Crosses conducted in summer 2012 for 'high sweetness'

Cross #	Seeds Produced	Pods Produced	Crossing attempts	Female parent	Male parent
12-732	3	1	3	DH618	CHOSENG NO.1
12-392	4	2	7	DH863	DH618
12-404	2	2	3	STARGAZER	DH618
12-418	3	1	5	DH618	DH748
12-417	1	1	5	DH618	HS09-144/1
12-663	4	2	13	DH618	KESZTHELYI APROSZEMU SARGA (A)
12-664	6	3	7	DH618	SUZUMARU

Table 4: Crosses conducted in a glasshouse at the University of Ottawa in the winter of 2012-2013.

Name	Female parent	Male parent
HS12-982	DH715L	DH863
HS12-976	DH863	PSX12C81S

In the summer of 2012, F1 plants that were the result of crossing in the winter of 2011-2012 were grown in Inkerman, ON. Some of these F1s contained DH863 (reduced beaniness) and DH618 (increased sweetness) in their pedigree. These F1 plants were harvested in the fall of 2012.

In the months of November 2012 to March 2013 the seed from the F1s that were harvested in Inkerman, ON was packaged and sent to Costa Rica (Table 5, Table 6). These populations were advanced two generations and then the F4 seed was returned to Inkerman, ON for spring planting in 2013.
The populations that are listed below will be evaluated for agronomic performance, visual seed quality and general impressions in the 2013 growing season. Individual varieties that arise from these populations will later be subjected to metabolomic screening using the identified biomarkers. Progeny will be selected for or against based on the outcome of this testing in subsequent generations.

Table 5: Plant material sent to Costa Rica for advancement of trait 'low beaniness'

NAME	PEDIGREE
HS11-367-1	DH863/(OT9814/IA1013)
HS11-367-2	DH863/(OT9814/IA1013)
HS11-368-2	DH863/KENJIANGDOU 43
HS11-368-4	DH863/KENJIANGDOU 43
HS11-368-5	DH863/KENJIANGDOU 43
HS11-368-7	DH863/KENJIANGDOU 43
HS11-368-8	DH863/KENJIANGDOU 43
HS11-368-9	DH863/KENJIANGDOU 43
HS11-372-1	DH863/PRIPYAT
HS11-372-1	DH863/PRIPYAT
HS11-372-2	DH863/PRIPYAT
HS11-372-2	DH863/PRIPYAT
HS11-372-2	DH863/PRIPYAT
HS11-519-2	DH863/213
HS11-519-2	DH863/213
HS11-519-4	DH863/213

HS11-519-4	DH863/213
HS11-519-6	DH863/213
HS11-577-1	OAC CALYPSO/DH863
HS11-577-10	OAC CALYPSO/DH863
HS11-577-11	OAC CALYPSO/DH863
HS11-577-12	OAC CALYPSO/DH863
HS11-577-2	OAC CALYPSO/DH863
HS11-577-3	OAC CALYPSO/DH863
HS11-577-4	OAC CALYPSO/DH863
HS11-577-5	OAC CALYPSO/DH863
HS11-577-6	OAC CALYPSO/DH863
HS11-577-8	OAC CALYPSO/DH863
HS11-577-9	OAC CALYPSO/DH863
HS11-591-2	0AR-73232 SCN/DH863
HS11-591-2	0AR-73232 SCN/DH863
HS11-591-3	0AR-73232 SCN/DH863
HS11-591-3	0AR-73232 SCN/DH863
HS11-591-4	0AR-73232 SCN/DH863
HS11-742-1	DH863/GD50344
HS11-742-2	DH863/GD50344
HS11-742-2	DH863/GD50344
HS11-742-3	DH863/GD50344
HS11-742-4	DH863/GD50344
HS11-744-1	DH863/(STARGAZER/Tanishi-PI243545)
HS11-744-10	DH863/(STARGAZER/Tanishi-PI243545)
HS11-744-3	DH863/(STARGAZER/Tanishi-PI243545)
HS11-744-4	DH863/(STARGAZER/Tanishi-PI243545)
HS11-744-5	DH863/(STARGAZER/Tanishi-PI243545)
HS11-744-6	DH863/(STARGAZER/Tanishi-PI243545)
HS11-352-2	SeCan 08-01C/DH863
HS11-352-2	SeCan 08-01C/DH863
HS11-352-2	SeCan 08-01C/DH863
HS11-352-2	SeCan 08-01C/DH863
HS11-352-2	SeCan 08-01C/DH863
HS11-676-1	DH748/DH863
HS11-676-2	DH748/DH863
HS11-676-3	DH748/DH863
HS11-676-4	DH748/DH863
HS11-676-5	DH748/DH863
HS11-727-1	SUINONG28/DH863
HS11-727-2	SUINONG28/DH863
HS11-727-4	SUINONG28/DH863
HS11-727-5	SUINONG28/DH863
HS11-727-6	SUINONG28/DH863
HS12-166-2	DH863/JACKSON
HS12-166-2	DH863/JACKSON
HS12-166-2	DH863/JACKSON
HS12-166-2	DH863/JACKSON
HS12-194-3	DH863/X790-P
HS12-194-3	DH863/X790-P

HS12-194-3	DH863/X790-P
HS12-194-3	DH863/X790-P
HS12-194-3	DH863/X790-P
HS12-181-1	DH863/P09-31/3LFLS
HS12-181-1	DH863/P09-31/3LFLS
HS12-181-1	DH863/P09-31/3LFLS
HS12-181-1	DH863/P09-31/3LFLS
HS12-181-1	DH863/P09-31/3LFLS
HS12-146-1	DH863/DH402
HS12-146-1	DH863/DH402
HS12-146-1	DH863/DH402
HS12-146-1	DH863/DH402
HS12-146-1	DH863/DH402
HS12-163-2	DH863/IA2101
HS12-163-6	DH863/IA2101
HS12-163-6	DH863/IA2101
HS12-171-1	DH863/LEO
HS12-171-1	DH863/LEO
HS12-171-1	DH863/LEO
HS12-171-2	DH863/LEO
HS12-171-2	DH863/LEO
HS12-148-3	DH863/DH530
HS12-148-3	DH863/DH530
HS12-150-1	DH863/DH715L
HS12-150-1	DH863/DH715L
HS12-150-1	DH863/DH715L
HS12-150-1	DH863/DH715L
HS12-150-1	DH863/DH715L
HS12-174-4	DH863/OAC CALYPSO

Table 6: Plant material sent to Costa Rica for advancement of trait 'high sweetness'

NAME	PEDIGREE
HS11-624-1	KESZTHELYI APROSZEMU SARGA (A)/DH618
HS11-624-2	KESZTHELYI APROSZEMU SARGA (A)/DH618
HS11-624-1	KESZTHELYI APROSZEMU SARGA (A)/DH618
HS11-624-2	KESZTHELYI APROSZEMU SARGA (A)/DH618
HS11-624-1	KESZTHELYI APROSZEMU SARGA (A)/DH618
HS11-655-1	PRIPYAT/DH618
HS11-655-2	PRIPYAT/DH618
HS11-655-2	PRIPYAT/DH618
HS11-655-3	PRIPYAT/DH618
HS11-756-1	(IA1017/ANGORA)/(DH618/213)
HS11-611-1	DH618/JIYU 94
HS11-611-1	DH618/JIYU 94
HS11-611-1	DH618/JIYU 94
HS11-611-4	DH618/JIYU 94

HS11-611-5	DH618/JIYU 94
HS11-572-3	RCAT 1003/DH618
HS11-572-3	RCAT 1003/DH618
HS11-572-3	RCAT 1003/DH618
HS11-572-3	RCAT 1003/DH618
HS11-572-3	RCAT 1003/DH618
HS11-656-1	HEINONG 38/DH618
HS11-656-2	HEINONG 38/DH618
HS11-656-3	HEINONG 38/DH618
HS11-656-4	HEINONG 38/DH618
HS11-656-5	HEINONG 38/DH618
HS11-656-6	HEINONG 38/DH618
HS12-343-1	DH618/JACKSON
HS12-343-2	DH618/JACKSON
HS12-343-2	DH618/JACKSON

Conclusions and Next Steps

The chosen tool (ultra HPLC MS QTOF) has proven its ability to determine differences between varieties grown in different locations. Next steps moving forward are to take the very large dataset resulting from this activity and interpret G x E effects on individual compounds. Further trials will be conducted to gather multiple years of analysis to further solidify findings.

The chosen tool has also proven its ability to identify parent seed with desirable biomarkers. Parent screening will continue for new parents entering into the Sevita International breeding program, so that they can be appropriately flagged - if necessary. Parents, that have been flagged to contain desirable biomarkers will continue being used in the breeding program and the resulting progeny will be sent for food testing to further confirm that the desirable food traits are present.

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

- Include the name of scientist / organization.

Plot Collaborators

McGill University - Philippe Seguin
Missouri Crop Improvement Association – Richard Arnett
Murphy et al Inc (Manitoba) – Keith Murphy
Kent AG Research Inc. – John Nevills
University of Saskatchewan Crop Research Center –Tom Warkentin
Sevita International Price Edward Island Research Station – Michelle Hickey
Don Mario Sementes – Ezequiel Pozzo

Sevita International

David Hendrick, CEO and Chairman of the Board
Jagdish Kumar, Plant Breeder
Richard Germain, Research Technician Manager
Dave McInnes, Research Technician
Dean Ward, Research/Crossing Technician
Dustin DeJong, Research Technician

Stacey Simpkin, Research Coordinator
Jim McCullagh, Vice President (Research)
Mark MacDuff, Trait Development Manager
Ronald Guillemette, Technical and Genetics Consultant

Sevita International Research Staff Members hired and trained during the AIP project:
Tylor Vezina, Assistant Research Technician, September 2012

Summer student/harvest help turned full time for some duration of the project:
Dennis Cooper, Research Technician
Alex Gravelle, Research Technician
Jill Kirkwood, Assistant Research Technician
Kimberly Ward, Assistant Research Technician

Summer students & harvest help, Assistant Research Technicians:
Erik dePater, Madison dePater, Laurena Matthies, Colleen O'Neil, Federico Cabacoy, Patrick Scharf, John Edwards

University of Ottawa
D. John T. Arnason - Professor
Ammar Saleem – Research Associate
Rui Liu – Laboratory Technician

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, 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.

Due to the amount of exploratory science required to identify the necessary compounds required for the project (Activity 2 & 3) and the short timeline of the project, Activity 4 activities were simplified and otherwise shortened to ensure that activities objectives were met (for example: fewer samples were sent for analysis than originally planned).

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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).

The impact of this is that the results for this activity will be further validated moving forward as further trials and analysis are undertaken in upcoming years.

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

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

An initial research paper on soybean metabolome is being prepared and other publications on applications are planned. All papers will feature Sevita International's germplasm.

F. Lessons learned (self-evaluation of project)

The scope of work under this activity was very broad for a short timeframe and thus can be improved upon through time with continued research. Future studies are required to provide multiple years of analysis, further validating techniques and results from the tool found in the ultra HPLC MS QTOF form of analysis.

Jim McCullagh		
PI Name	**Date**	**Signature**

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