Edamame Seed Saving and Genetic Diversity Study

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Executive Summary

Previous studies on vegetable soybean "Edamame" (Glycine max l. Merrill) on varietal adaptation and organic management under reduced irrigation have identified suitable varieties for cultivation in lower mainland metro Vancouver and other pockets of BC with similar agro-climate. In addition, seed color variants obtained in the field were identified as natural mutants and grown for generation advance creating larger gene pool of diverse breeding lines. These lines were segregating for various traits including seed color. Seeds of promising varieties and breeding lines harvested in 2013 and stored under room temperature were available for further study in 2015. With the doubt of seed viability and limited seed stock, "Seed Saving and Genetic Diversity Study" was planned as volunteer activity at the Sharing Farm, Richmond. Eight adapted varieties, SPS-BL-3, SPS-Isophenic, SPS-BL-1, Haruno-Mai, Sayamusume WCS, Sayamusume, TSC, Beer Friend and Shirofumi and four mutant populations; M_2 of SPS-UBC-Isophenic, M_{3s} of SPS-Isophenic, SPS-BL-3, and SPS-BL-1 were set as study materials. The main objective was to study status of seed viability, germination, seedling growth, plant development, yield performance and also selection of promising breeding lines for further testing. Study materials were planted on May 28, 2015 on 100 ft long and 3 ft wide well prepared beds at the Sharing Farm. Seeds were dibbled at normal 5-6 seeds/ft for adapted varieties whose germination was tested and it was thicker for mutants population whose germination was not tested. The drip irrigation was applied and plots were manually weeded. Regular visit was made to record the growth and development of crop from germination to harvest. In vitro germination test indicated varietal difference in viability and seedling growth among adapted varieties. Seedling emerged in 7-10 days in the field showing clear differences in seedling health within and between varieties. Sayamusume, TSC and Shirofumi composite seed stock had very poor germination while others had satisfactory level of seedling establishment. Seedlings of Sayamusume WCS and M_3 of SPS-BL-3 developed diseases and died out as growth stage progressed and surviving plants growth was deformed because of mosaic and green plant syndrome development. All four mutant populations exhibited wide genetic diversity with high value for selection in order to variety development possessing seed color different from the parental stock. Seed borne and soil borne disease can reduce seedling growth and can result into complete crop failure. Data recorded at various stages indicated that the crop care during harvest is vital for saving a quality seed. Study provided further evidence that quality seed of edamame can be produced locally in order to bring edamame into commercial cultivation with large beans yielding about 20 tons fresh organic green pods that could be brought in the local market. The present problem is the lack of organization bringing research and development into academic and promoting local production.

Edamame Seed Saving and Genetic Diversity Study

Background

Vegetable soybean (*Glycine max Merr L.*) popularly known as Edamame, is a crop with rising popularity in the western world. Its rich nutritive value and health benefits have created increasing market demand among health conscious consumers. Though this crop is generally adapted to Asian countries, its cultivation is slowly expanding in Europe and North America. Edamame seeds and frozen pods imported from Asia, mainly from China, Taiwanand Japan, are available in market place and super stores. However, edamame grown in small area in different parts of USA and Canada are still not able to reach in frozen section of super stores. Atlantic North East and Pacific North West regions are suitable for edamame cultivation but there is no official record of promotional efforts for its cultivation in BC.

Richmond Food Security Society and The Sharing Farm Society supported the edamame study focused on adaptation testing and varietal development from 2009-2013. Two varieties (one yellow seeded labeled as Edamame and other as Black Jet) received from Dan Jason of Salt Spring Seeds Inc, were planted in 2009 to test the possibility of adaptation. Results encouraged to make seed increase planting in long rows in 2010 and close observation identified three individual promising plants under marginal fertility and reduced irrigation. They were harvested separately and labeled as Single Plant Selection Breeding Line 1, 2 and 3 (SPS-BL-1, SPS-BL-2 and SPS-BL-3). Two previous varieties plus three breeding lines and five additional varieties received from Anapolis Seeds Inc of Nova Scotia, were planted in 2011. Results were promising and a fully fledged adaptation study in 2012 and yield stability study in 2013 were conducted with the grant support of Organic Sector Development Program (OSDP) administered by Certified Organic Association of British Columbia (COABC). These studies created many segregating lines through natural mutations and also identified few promising varieties (genotypes) for commercial cultivation in few suitable pockets of BC. However, good seed quality, proper soil health (temperature, moisture, pH, fertility and presence of beneficial and absence of harmful soil micro-organism) and sunny days towards maturity were identified as critical for successful production in Lower mainland of BC.

Varieties such as Sayamusume WCS, Sayamusume TSC, Beer Friend, SPS-BL-3, SPS-Isophenic, Haruno-Mai, and Shirofumi performed satisfactory, yielding green pods as high as 20 tons/hectare. Segregating seed color mutants originated from SPS-BL-1, SPS-BL-2

(Isophenic) and SPS-BL-3 in 2012 with variable seed color (black, brown, beize and yellow), seed weight (as high as 50 (g) to as low as 20 (g) per 100 seeds) and variable plant morphological and maturity traits differences are valuable genetic pool for further research and development of edamame. The promising varieties and mutant breeding lines are under study at UBC farm. However, direct field planting quality seed saving is a challenge as crop maturity coincides with wet and cold humid weather at Northwest Pacific regions. Therefore, comparative seed quality evaluation and genotype selection as well as innovation study for quality seed production techniques are needed as further steps towards promoting edamame production under Northwest Pacific climate. Therefore, this study was designed to critically:

i**) observe the differences in germination and seedlings growth between and within variety and interpret the main cause for the difference,**

ii) observe the differences in growth and development of surviving seedlings to attain healthy vegetative, reproductive and adult stage and interpret the differences with scientific logic,

iii) practice the seed saving activity during seed maturity even under humid weather without possible pod infection by saprophytic micro-organism, and

iv) select promising lines of segregating mutants and explore the potential source to conserve and utilize valuable genetic resources that are created at the Sharing Farm.

Materials and Methods

Study materials: Eight varieties of edamame that were verified as promising varieties for cultivation in suitable agro-climatic pockets of BC (Table 1) were included in seed saving study. The seed color segregating mutant populations with wide genetic diversity and potentiality to develop into a promising variety to adapt climate change were also included in the study (Table 2).

Table 1. List of varieties included in study, source of seed and flower, seed color and morphology

Variety	Source of Seed	Flower and Seed Character
SPS-BL- 3	Sharing Farm (Salt Spring Seed)	White flower, large size yellow seed
SPS-Isophenic	Sharing Farm (Salt Spring Seed)	White flower, medium large size yellow seed
SPS-BL-1	Sharing Farm (Salt Spring Seed)	White flower, medium large size yellow seed
Haruno- Mai	Sharing Farm (Salt Spring Seed)	White –cream flower, medium size yellow seed
Sayamusume WCS	Sharing Farm (West Coast Seed)	White flower medium size yellow seed- short plant
Sayamusume TSC	Sharing Farm (Territorial Seed)	White flower, medium size yellow seed- tall plant
Beer Friend	UBC Farm	White flower, medium size yellow seed
Shirofumi random	Sharing Farm (Anapolis Seed)	White flower, medium size yellow seed
Shirofumi SPS	Sharing Farm Select (Anapolis Seed)	White flower, medium size yellow seed

Note: Information within parenthesis indicates the original source of seed. SPS- BL= Single Plant Selection –Breeding Line, selected from population grown at Sharing Farm in 2010.

Seed stock harvested and threshed during wet weather in 2013, and stored under ordinary room temperature was available for planting in 2015 summer. As the crop harvest in 2013 was coincided with wet and cool temperature, crop could not be threshed under dry condition and seed moisture content was higher than desired level for safe storage. Therefore, seed viability was a question and germination test was needed. Germination test was run in April 2015 which indicated various degrees of germination ability though seed viability of 2013 stock was high. However, the older stock from 2012 lost total viability except Black Jet, a variety with black coat color which retained about 2% viability (Table 3).

Table 2. List of segregating populations, source of the origin and seed coat color of mutant edamame.

Population	Pedigree	Parent - Color
M_2 SPS-UBC-Isophenic	M_1 plant identified with brown seed at UBC 2013	SPS-BL-2 - Yellow
M_3 SPS-BL-3	M_2 grown in 2013 at Sharing Farm- segregating	SPS-BL-3 - Yellow
M_3 SPS-Isophenic	M_2 grown in 2013 at Sharing Farm- segregating	SPS-BL-2- Yellow
M_3 SPS-BL-1	M_2 grown in 2013 at Sharing farm- segregating	SPS-BL-1 - Yellow

Table 3. Viability and seed germination of edamame seed stocks of 2012 and 2013 in April 2015.

Genotype	Stock year	Germination (%)			Genotype	Stock year	Germination (%)		
		A	B	C			A	B	C
SPS-BL-3	2013	20	40	40	Beer Friend	2013	0	90	10
SPS-Isophenic	2013	30	30	40	Shirofumi	2013	10	20	70
SPS-BL-1	2013	30	50	20	Shirofumi	2012	0	0	100
Haruno-Mai	2013	30	30	40	SPS-BL-3	2012	0	0	100
Sayamusume WCS	2013	40	30	30	Hakucha	2012	0	20	80
Sayamusume TSC	2013	0	70	30	SPS-Isphenic	2012	0	0	100

A=seedlings longer than 9cm and B= Seedlings shorter than 8cm and C= Total not germinated.

Results from germination test were used to adjust the seeding rate accordingly based on seedlings count that could support the growth to develop into healthy plant. Growth of embryos, seedlings and seed-borne airborne pathogen could be seen affecting the growth of seedlings (Fig 1). Promising looking single plant from Sayamusume WCS and Shirofumi were selected and air dried by hanging under the sun in 2013. Seed moisture content seemed relatively lower than that of composite stocks which were not air dried. Germination of these seeds was not tested because of seed limitation and presumably better seed viability due to lower seed moisture content.

Segregating seed coat color mutant populations (Table 2) were second set of materials for genetic diversity and seed saving study. However, seed viability and germination of these materials was not tested because of limited seeds. Selected good quality looking M_3 seeds of MBL-3 (beize color seeds composite of 15 M_2 plants), MBL-Isophenic (brown color seeds composite of 15 M_2 plants) and MBL-1 (black color seeds composite of 8 M_2 plants and brown color seeds composite of 8 M_2 plants), and 69 M_2 seeds of SPS-UBC-Isophenic were the materials for the study.

Field preparation and planting: Field was plowed, composted and added organic manure to maintain soil fertility. Compost and manure was plowed under the soil and 100 feet long 3 feet wide two beds were raised for seed saving study. Drip irrigation was installed to better manage irrigation. Eight adapted varieties were planted in May 28, 2015 by dibbling the seeds in 3 feet long rows spaced 18 inch apart. Number of

rows planted for each variety was different based on amount of seed available and ability to germinate (Table 4). Effort was made to maintain 3-4 plants per foot. Segregating M_2 and M_3 populations were planted in two long parallel rows spaced 18 inch apart. Length of the rows was determined by the amount of seeds available for dibbling. Seeds were dibbled thickly in May 28, 2015 as the viability was not tested.

Crop management and field data recording: Regular hand weeding was done as and when needed. Water on the pipe line was turned on every morning for two hours. This summer was exceptionally drier than previous years and drip irrigation was proper for water management. The system was turned off when the crop was reaching physiological maturity in order to create drier micro-climate. Germination, plant stand and growth and development were monitored regularly by close observation and data on vegetative and reproductive growth including disease development was recorded on standing plants. Six plants from each entry were harvested to record plant height, branches per plant, nodes on the main stem, total pods on plant, pod types (1-bean pod, 2-bean pod and 3-bean pod). Pods from individual six plants were bulked threshed and averaged to represent as single plant. Total of twenty 3-bean pods from each genotype were selected for data on fresh weight of pods. Remaining plants were harvested as and when pods attained maturity at different dates. The first harvest was on September 16, and last was on October 10, 2015. Pods and seeds were sun dried and weight of selected 20 seeds for eight genotypes and selected 10 seeds of segregating population was recorded and expressed as 100 dry seed weight (g).

Data analyses and report preparation: In absence of replicated observation on adapted varieties and segregating populations, data was recorded on individual plant basis and presented as mean values. Range, standard deviation and correlation were calculated and presented for segregating populations. Photograph of the seed saving study was taken at various stages of plant development to depict the true picture in the field. Some of these photographs are used in the report for discussion and result interpretation. Mean data are presented as Tables in the main text and detail data on individual plants of segregating population is presented as Appendix.

Result Presentation

Result of seed viability and germination test is presented below (Table 3 and Figure 1). Seed stocks from 2012 lost 100% viability except Hakucha with 20% viability and were not included in the study. Germination was as low as 30% for Shirofumi to 90% for Haruno-Mai. The late maturing varieties like Sayamusume TSC, Shirofumi Sayamusume WCS and SPS-BL-3 had fewer well grown healthy seedlings than SPS-Isophenic, BL-1, Haruno-Mai and Beer Friend. Infected seedlings die out in struggle to survive.

Figure 1. In vitro and in vivo seed germination and seedling establishment of edamame varieties during spring season 2015.

Seedlings emergence in the field Figure 1 (right) confirmed the observation of left photo based on the fact of poor emergence and plant stand of varieties Sayamusume TSC and Shirofumi planted from composite seed stock. Plant stand of from selected plants of Shirofumi and Sayamusume WCS was satisfactory. Number of rows planted is presented in Table 4. Six rows of Single Plant Selection of Sayamusume WCS and five rows of Single Plant Selection of Shirofumi germinated well to maintain normal plant stand.

Figure 2. Seed germination and plant population variation within and between rows in addition to varietal difference contributed to seed quality. Blank row on the right is because of zero germination of slected SPS-Isophenic that was accidently cut before embryo maturity.

There was clear differences in number of seedlins, seedlings health and vigor among varieties and rows within variety. This indicated variation in individual seed quality within and between varieties. This is clearly shown in Figure 2. Wide gap between plants within row and wider gap between six rows and or 3 rows indicated seed quality difference within variety and between varieties, respectively (Figure 2 right). Plant stand of M_3 populations of SPS-BL-3, SPS-isophenic and SPS-BL-1 was good because of thicker seeding.

Table 4. Number of rows of adapted edamame varieties planted for seed saving study in 2015.

Genotype	**Rows planted (#)**	**Genotype**	**Rows planted (#)**
SPS-BL-3	4 + 3 Composite =7	Sayamusume WCS	6 (Composite) + 6 (SPS)** =12
SPS-Isophenic	3 + 2 Composite =5	Sayamusume TSC*	6 Composite =6
SPS-BL-1	3 Comp;osite =3	Beer Friend	6 + 6 Composite =12
Haruno-Mai	6 + 3 Composite =9	Shirofumi	3 (Composite)* + 5 (SPS)**=8

***, indicates very poor plant stand while ** indicates normal plant stand**

Figure 3. Plant growth and foliage color differences between two rows of the same genotype due to nutrient deficiency (left) and natural natural recovery after few weeks of growth and development in edamame during 2015.

Seedlings infected with seed and soilborne disease looked weak showing pattern of abnormal growth right from the germination and emergence stage. Many of the seedlings struggling to catch normal growth died and remaining plants produced branches to compensate the open space with the canopy development. Mosaic of leaf color pigmentation was observed which was attributed to nutrient deficiency, mainly nitrogen (Figure 3). Plants recovered from nutrient deficiency at later stage of vevegative development through nitrogen fixation and mineralization and attained canopy closure by branching as much as possible.

Figure 4. Poor plant population (left), canopy growth (plant coverage) and disease development progressed (right) towards reproductive development of varieties with poor quality seeds during 2015.

Results of poor seed and diseased seedling on over all plant growth and development from vegetative to reproductive stage is depticted in Figure 4. Front portion of the two row left bed is M_3 plants of SPS-BL-3 and first six rows and big gap on the right of each photos are of Sayamusume WCS and Sayamusume TSC, respectively. Bacterial and fungal and viral diseases slowly developed on the entire foliage including stem causing abnormal growth reflecting each disease organism. Reproductive growth of M3 SPS-BL-3 and Sayamusume WCS (composite) was severly affected except few disease free plants (Figure 4 right). Sun burn injury was noted which recovered after plant atained adult stage. Few major diseases noted in the field during the entire growing period are presented in Figure 5 and Figure 6. All these major diseases are seed as well as soilborne as seed and soil treatment was not performed.

Figure 5. Soybean mosaic virus (left), bean pod mottle virus (centre) and Cercospora leaf blight (right) infection in edamame grown during 2015.

Figure 6. Bacterial blight plus soybean mosaic (left), Cercospora leaf and pod blight (centre) and Sclerotinia stem rot (right) occurrence in edamame grown during 2015.

Soybean mosaic virus, bean pod mottle virus (plant mottling and also green stem syndrome), Cercospora leaf and pod blight, Sclerotia stem rot were among major disease affecting healthy vegetative and reproductive growth of edamame. These organisms could be pod as well as seed borne if crop maturity coincides with cool humid weather favorable for disease growth on the pods before and after harvesting.

Table 5. Variation in plant growth and development within and between edamame varieties grown at Sharing Farm, Richmond during 2015.

Varieties	Plant stand	Branch (#)	Stem nodes (#)	Anthesis days (#)	Canopy height (cm)	Plant height (cm)	Pod maturity (days)
SPS-BL-3	Good	1-2	11 (09-13)	46	60	50 (34-62)	95
SPS- Isophenic	Good	1-3	09 (08-11)	46	58	37 (25-56)	95
SPS-BL-1	Good	1-2	11 (09-13)	46	49	35 (18-56)	95
Haruno-Mai	Good	1-2	11 (10-14)	40	71	61 (40-70)	90
Sayamusume WCS	Good	1-3	10 (08-13)	40	50	46 (25-65)	95
Sayamusume TSC	Poor	3-4	11 (09-13)	49	56	30 (23-370	105
Beer Friend	Good	1-2	09 (08-12)	40	53	47 (35-68)	95
Shirofumi composite	Poor	3-4	10 (08-13)	54	46	26 (18-35)	110
Shirofumi-SPS	Good	1-2	14 (11-16)	54	71	58 (40-68)	110

Pattern of branching, nodes and inter-node development varied with available growth space, health of the seedling and genotype in adapted varieties as well as in segregating mutant populations (Table 5, 7 and Appendices I, II, III, IV). Wide range in growth parameters was observed between plants of same varieties. Although some degree of variation was observed among varieties in branching, nodes formation and plant height, space available for plant growth played major role in decision making (Figure 7). Plants under thick stand showed tendency of not branching and instead grew taller with more nodes on main stem. Plants in poor stand produced many more branches and few nodes on the main stem with more number of nodes on the plant making it bushy. This was supported by the data of Shirofumi composite and Shirofumi SPS (Table 5 and Figure 8). Diseased plant produced side branch to bear pods as it might have sensed the inability to bear pods on main stem (Figure 7 left).

Figure 7. Plant canopy coverage and stand compensation (left) and maturity differences (right) among genotypes of edamame in 2015.

Figure 8. Abnormal branching and pod bearing (left), normal (centre) and profused branching and pod bearing (right) in edamame in 2015.

Days to anthesis ranged from 40 to 54 amnog adapted varieties and segregating mutant lines. Full bean developed green pods were ready for harvesting in 90 to 110 days (Table 5 and Figures 7, 8 and 9).

Figure 9. Patterns of branching (Sayamusume WCS left and Sayamusume TSC centre) under poor plant stand and normal branching under normal plant stand (right) in edamame grown during 2015.

Number of pods produced on plant ranged from as low as 12 for Sayamusume WCS to as high as 93 for Sayamusume TSC (Table 6). Sayamusume WCS showed satisfactory level of initial plant stand but it later exhibited deformed vegetative and reproductive growth and many plants did not produce pod at all. Green plant syndrome was common which was caused by seedborne bean mottle virus. Sayamusume TSC composite seed had the poorest plant stand and few surviving healthy seedlings that attained profuse vegetative growth and produced pods as high as 93. Weight of 20 selected 3-bean pods ranged from 86 gm for Haruno-Mai to 111 gm for SPS-BL-3 followed by 104 gm for Shirofumi. SPS-Isophenic and Sayamusume TSC produced higher percentage of 2 and 3- bean type pods combined (Table 6). Dry seed weight (gm)/100 ranged from 48 to 54 and 39 to 47 for selected and random seeds, respectively. Difference between selected and random dry seed weight for each varieties was within the similar range of 7-9 gm. Total seed yield (gm) was variable among varieties which was determined mainly by the health of individual plant, its productive ability and number of plants. The mean, standaard deviation and ranges for important parameters measured in four segregating mutant population is presented in Table 7 and detail data is presented in Appendix I-IV. Plants producing more than 80 pods with large seeds of different colors weighing more than 48 (gm)/100 seeds have been identified. These are promising lines for further evaluation and varietal development. A total of 13 breeding lines of M_2 SPS-UBC-Isophenic (UBC1501 to UBC1508 and UBC 1510 to

UBC1513 and UBC1516) were identified . Four breeding lines of M_3 SPS-BL-3 (MBL31501, MBL31503, MBL31508, and MBL31513) were identified. Thirteen breeding lines of M_3 SPS-BL-1 (MBL11501, MBL11504, MBL1506, MBL11507, MBL11510, MBL11512, MBL11515, MBL11517, MBL11519, MBL11520, MBL11522, MBL11555 and MBL11565) were selected for further study. Twelve breeding lines of M_3 SPS-Isophenic(MBL-I-15-01, MBL-I-04, MBL-I-05, MBL-I-06, MBL-I-07, MBL-I-10, MBL-I-11, MBL-I-12, MBL-I-14, MBL-I-24, MBL-I-27, MBL-I-51) were also identified. All selected bteeding lines are marked with bold face.

Table 6. Variation in pod and seed yield components within and between edamame varieties grown at Sharing Farm, Richmond during 2015.

Varieties	Planted Rows (#)	Pods/plant (#)	3-bean wt. (gm)/20 pod	1-bean pods (%)	2-bean pods (%)	3-bean pods (%)	S-Seed wt (gm)/100	R –Seed wt (gm)/100	Total seed yield (gm)
SPS-BL-3	7	49 (32-68)	111	23 (14-34)	43 (41-58)	29 (16-40)	52	44	837
SPS- Isophenic	5	43 (30-68)	104	17 (11-23)	48 (27-59)	35 (25-51)	49	41	451
SPS-BL-1	3	50 (31-65)	100	21 (13-38)	15 (32-68)	28 (19-37)	53	44	438
Haruno-Mai	9	49 (27-67)	086	33 (21-67)	45 (30-55)	32 (13-63)	48	39	997
Sayamusume WCS	12	31 (12-47)	100	35 (12-47)	46 (36-56)	19 (11-36)	54	45	663
Sayamusume TSC	6	70 (46-93)	102	17 (12-22)	66 (63-69)	17 (09-25)	53	47	492
Beer Friend	12	31 (21-52)	100	30 (19-70)	48 (26-67)	16 (04-36)	50	43	743
Shirofumi comp	3	53 (28-89)	106	19 (18-21)	58 (53-64)	22 (18-26)	54	45	325
Shirofumi-SPS	5	61 (37-71)	-	32 (23-42)	54 (45-69)	14 (06-21)	48	39	730

Correlation coefficients for few traits of importance within the scope of this study of segregating population with different observation number 29 for M_2 SPS-UBC-Isophenic , 69 for M3 SPS-Isophenic, 80 for M3 SSP-BL-1, and 15 for SPS-BL-3 are presented (Table 8). Correlation coefficients between characters were in the same direction but varied in degree. The correlation was weaker in SPS-BL-3 and stronger in SPS - UBC-isophenic. It was followed by SPS-BL-1 and SPS-isophenic. The correlation between plant height and branch was positive but lower than for othe traits. This indicated that plabt height and branch do no posses strong tie in this study. Plants that grow bushy under thin population produce many more branches than grown under normal thicher plant stand. The correlation between plant height and branch +0.59), number of pods per plant and plant height (+0. 68), pod yield and nodes per plant (+0.80) and seed yield and 100 seed weight (+0.59) is suggesting a possibility of identifying desirable line for variety development. Correlation between plant height, branch and node with pod type (1-bean, 2-bean and 3-bean pod) was not significant. Similarly, the correlation of pod yield and seed weight with pod type was poor and variable suggesting no need to worry about these components' relationship among these varieties.

Table 7. Mean and standard deviation for few traits of segregating mutants of edamame grown at Sharing Farm during 2015.

Population	**M_2 SPS-UBC- ISOPHENIC**			**M_3 SPS-ISOPHENIC**			**M_3 SPS-BL-1**			**M_3 SPS-BL-3**		
Variables	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Plht (cm)	33.9	10.5	17-52	46.0	22.7	13-105	33.8	11.1	9-55	22.6	8.3	10-36
Branch (#)	1.7	1.0	0-3	1.2	0.8	0-3	2.0	1.0	0-4	1.3	0.8	0-3
Node (#)	9.4	2.3	7-15	10.4	3.4	5-17	8.5	1.6	5-13	8.8	2.3	6-14
Pods/pl (#)	37.7	28.9	8-105	24.7	19.7	6-84	28.3	17.8	9-92	13.9	8.0	5-33
2-bean (%)	51.3	12.1	30-80	48.4	12.9	17-75	47.7	12.3	20-81	53.0	14.6	30-83
3-bean (%)	24.5	9.5	4-38	20.1	12.3	0-50	22.3	11.7	0-63	26.4	13.0	0-50
Seedyld (gm)	26.3	19.8	5-63	14.5	10.7	4-49	16.9	11.9	4-70	9.7	4.8	2-18
SW/100 (gm)	40.8	10.2	20-52	33.3	8.7	20-50	35.5	9.4	20-52	36.3	11.0	15-50
Total plant (#)	29			69			80			15		

Seed quality concerns are depicted in Figures 9, 10 and 11. Some pods infected by Cercospora leaf and pod blight contained stained seeds but very little. Shirofumi, SPS-BL-1, SPS-BL-3 and Haruno-Mai had more cracked seeds than others. Seed cracking was partly attributed to plumpness of beans which would be desirable for green pods used as edamame. Seeds were healthy and only few were cracked in adapted varieties. Twentynine M_2 of SPS-UBC-Isophenic produced all brown color seeds which were categorized into solid brown=19 and cracked brown =10. Average dry seed weight ranged from 20 -52 (gm) with average of 40 (gm)/100 seeds. Similarly, 69 M_3 plants of SPS- Isophenic produced brown solid and brown cracked seeds having a weight range from 20-50 (gm)/100 seeds. Fifteen plants of M_3 SPS-BL-3 produced yellow, beize, and black color seeds with weight range from 15-50 (gm)/100 seeds. Seeds from 80 M_3 plants of SPS-BL-1 population segregated for seed size ranging from 20-52 (gm)/100 seeds with solid and cracked seeds of black, brown and yellow color. Seed cracking observed in segregating lines is genetically controlled and indicated seed coat softness compared to solid hard seed coat. Genotypes with cracked seed coat might posses some nutritional advantage.

Table 8. Correlation coefficients between traits contributing to number of pods and seed yield (gm) per plant in 2015.

➢ **Population Variable**	**M_2 SPS-UBC-ISOPHENIC**	**M_3 SPS-ISOPHENIC**	**M_3 SPS-BL-1**	**M_3 SPS-BL-3**
Pods (#) & Plht (cm)	0.68	0.62	0.41	0.36
Pods (#) & Branch(#)	0.70	0.42	0.63	0.27
Pods (#) & Node (#)	0.78	0.69	0.56	0.42
Pods (#) & SW (gm)	0.49	0.30	0.14	0.12
Seedyld (gm) & Plht	0.66	0.61	0.36	0.04
Seedyld (gm) &Branch	0.75	0.41	0.57	0.19
Seedyld (g) & Node	0.80	0.66	0.44	0.07
Seedyld (g) & SW	0.59	0.43	0.45	0.41
Plants observed (#)	**29**	**69**	**80**	**15**

Figure 9. Seeds of eight varieties of edamame (left) and M_2 of SPS-UBC-Isophenic population producing solid brown seeds (19 plants) and cracked brown seeds (10 plants) at the Sharing Farm during 2015.

The brown edamame seemed to posses' softer seed coat and looked more plump than parental yellow color edamame. Most of the plants with cracked seeds produced few pods with seeds.

Figure 10. Seeds from M3 population of SPS-Isophenic (left) segregating for seed size and color, brown solid 57 plants (variable seed size) and brown cracked 17 plants (variable seed size) plus seeds from 15 M_3 plants of SPS-BL-3 segregating for seed quality (color, seed size). Fifty two plants of SPS-Isophenic population were grouped into two seed stocks as large seeded and small seeded. Large seeded ones were darker brown as opposed to lighter brown of small seeded ones. Green stem syndrome resulted into production of deformed and infected decaying seeds in SPS-BL-3 lines.

Figure 11. Parental SPS-BL-1 and its M1 (grown in 2012) and its M3 segregating into solid black seeds (48 plants), cracked black seeds (17 plants), solid brown seeds (10 plants), cracked brown seeds (8 plants) and yellow beize color seeds (3 plants) grown at the Sharing Farm during 2015. Breeding lines with large seeds with black, brown and beize seed coat looked attractive if supported by the capacity of producing more pods per plant in comparative analysis.

Discussion and Conclusion

Climate change has become the serious challenge for human welfare and sustainable livelihood in 21st century. Agriculture is a sector strongly affected by changing trend of agro-climate at local and global level. Therefore, climate - smart agriculture has emerged as a proper concept and strategy to adapt climate change for environmental conservation, food security, and agricultural sustainability capable of feeding the world population. Health and environmental issues of GMO have resulted in stepping down on the application of GMO technology by the farmers that was once thought as a possible way of feeding the world population. Agriculture production based on natural inputs, such as organic resilient method, and use of locally produced seeds of adapted high yielding varieties are emerging as environmental friendly sustainable alternatives. Conservation of existing genetic resources, identification of new genes naturally created as mutants that adapt climate change under field condition, their conservation, evaluation and use, are important activities. Seed saving and quality seed production at local level deserve special attention in order to address the issues of local food production system and food security.

Quality seed production requires drier climate during crop maturity and seed harvest to avoid seed infection. The weather of lower mainland of BC including Richmond is generally wet and unfavorable for quality seed production of edamame. The focus of research and promotion of edamame at Sharing Farm in 2015 was on study of old seed stocks on germination, emergence, stand establishment and vegetative and reproductive growth and development. Based on the in vito and in vivo results, quality seed production and storage in Vancouver climate is possible but needs extra attention at harvest period. Seed viability and germination is not affected much by the presence of seed borne pathogens but it declines sharply if seed moisture content is high to activate pathogens. Seed lot harvested in 2012 lost seed viability because of the same reason. Seed lot from 2013 harvest possessed different levels of seed moisture content among seeds of the same as well as different varieties and exhibited the true picture of each seed based on quality. Individual seed quality affected the performance right from the seed germination, seedling health (growth, strength and pace of development, plant stand, branching, canopy growth, plant height, disease development, pod production (number of pod and pod type; 1, 2, 3 bean pods), seed development, seed weight per pod and grain yield per plant. Adapted varieties had only limited seeds for planting 3-12 rows and could not be thickly seeded like segregating population (M_3, SPS-BL-3, SPS-BL- Isophenic and SPS-BL -1). M_2 SPS-UBC-isophenic had only 59 seeds and 29 healthy plants completed the physiological maturity. Other two populations that were seeded thick maintained thicker plant stand compared to adapted varieties that were planted thinner.

Satisfactory germination and initial seedling stand of Sayamusume WCS later turned into the poorest performer because of multiple disease development causing stunting, leaf puckering, curling, stem thickening and abnormal vegetative growth that prevented reproductive growth. Sayamusume TSC lost seedling strength and could not emerge and few crawling seedlings died resulting into the poorest plant stand development. Among the survival plants, few were healthy and disease free but many of the other were disease affected and failed to produce seeds. The plants from a healthy and a poor seed present the true picture highlighting the value of quality seed saving (Figure 10). Both plants produced branches to compensate the poor stand but the plant developed from poor seed could not succeed to enter reproductive growth. Therefore, seed health decided the fate of growth and development.

Figure10. Result of seed quality differences (left) bean pod mottle virus infected and healthy plant (right) of edamame.

Seed quality differences in different lots of 2013, for example, Shirofumi composite v/s Shirofumi SPS (Single Plant Selection), and Saymusume WCS composite v/s Sayamusume TSC SPS(Single Plant Selection) resulted because of difference of plant handling. In general, the harvesting of edamame was during wet weather and lacked proper drying, threshing and handling of seeds before and during storage. This problem could be solved by paying proper attention while harvesting for seed. For example, SPS were harvested and plants were air/sun dried and dry pods were threshed which produced dry and good quality seeds. They retained viability, germination and seedling strength for emergence better than composite seed lots of the same variety. This could be easily done in hoop or glass house with air dry facility for seed drying and saving.

Drip irrigation supported the plant growth during entire dry period with minimum water. Different foliar disease such and soybean mosaic virus, bean pod mottle virus causing "Green Stem Syndrome", Cercospora leaf and pod blight, Sclerotia stem rot, bacterial blight, bacterial pustule and bacterial tan spot infested the crop from seed and soil source. These diseases could be prevented if organic seed treatment is practiced. Therefore, these diseases could not be considered as threat for seed production. Nutrient deficiency showing symptoms similar to nitrogen few weeks after emergence was naturally recovered probably by nitrogen fixation and other nutrient mobilization in association of soil microbes. The performance of healthy plant of all adapted varieties was encouraging by exceeding even the potential pod yield of 20 tons/hectare report in 2013 at the Sharing Farm. Detail information of these varieties under various studies from 2010 to 2013 is available at the Sharing Farm website. In 2015, healthy large seeds were harvested from the field by picking dry pods and threshed after sun and air drying to produce quality seeds. However, this is not practical for large farm.

Segregating M2 and M3 populations are valuable as they posses different color seed coat, brown, black and beize that is different from original parent. Some of the breeding lines are very promising with respect to seed size, pod yield and seed yield. The mean value and highest value for M_2 SPS-UBC-Isophenic, M_3 SPS-Isophenic, M_3 SPS-BL-1 and M_3 SPS-BL-3 presented in Table 7 indicated a clear message that generation advance and selection from breeding lines can generate edamame varieties that are different in color with potential high yield. M_2 SPS-UBC-Isophenic and M_3 SPS-Isophenic had all brown seeds different from yellow seeds of parent SPS-Isophenic. M_3 SPS-BL-3 has beize (MBL31504) and brown (MBL31501) seed color different from yellow seed of the parent SPS-BL-3. Similarly, M3 SPS-BL-1 has black (MBL11501, MBL11504, MBL11507, nMBL11510, MBL11515, MBL11517, MBL11519, MBL11519, MBL11520, MBL11522 and MBL11555), brown (Mbl11506, MBL11512 and MBL11565) seed color different from yellow parent SPS-BL-1. Selected breeding lines with the same color within a population could be composited and planted for generation advance and selection during summer of 2016.

Based on the results and discussion, the specific objectives are well addressed. The differences in germination and seedling growth within and between varieties are attributed to seed quality differences among individual seeds which were exposed to variable temperature and moisture regime during harvesting and threshing. The wet weather towards crop maturity was favorable for pod and seed infection and demanded extra efforts to create healthy and safe seed storage which was not available during 2013. This problem for quality seed production and saving to preserve seed viability, can be resolved without much budgetary difficulties at the research stations and farms. Organic treatment of soil and seed before planting can prevent disease development and promote germination and healthy seedling development.

Germinated seeds that emerged from the soil showed various seedling health status. Some seedlings were affected by multiple disease complexes that struggled to survive and grow but ultimately died out. Few seedlings were healthy and grew and attained profuse vegetative and reproductive development. Low plant population and available open space for branching resulted into bushy shorter plant. These plants possessed many nodes on the whole plants and produced many pods right from the lower section of the canopy which was still open. Varieties and /or rows with recommended plant population (5-6 plants/ft). Narrow spacing between plants discouraged the branching and plants grew taller closing the canopy before anthesis. There was tendency of minimizing pod formation at the lower section and maximizing at center and top section of the canopy. Plants stayed green longer when canopy remained open compared to the plants with closed canopy. Though branching compensated low plant stand to certain degree, normal plant stand is desirable when weed and crop management is considered. However, seed size and pod type is more determined by the genotype.

Since the study was focused on quality seed production and saving, every effort was made to create drier micro-environment around plant canopy. Drip irrigation system was cut out as soon as bean on the pods were fully developed approaching physiological maturity. Dry pods that attained matured adult (past yellow and pod shell drying) phase, were picked individually and/or whole plant was harvested and dry pods were picked. All pods were dried under the sun and threshed. Plants with full developed green pods just turning yellow were harvested and let the pods turn complete yellow and seed dry by hanging under shade where air and sunlight could dry the pods. Growth of saprophytic pathogen was restricted because of drier pods which prevented seed infection.

The seed were dried under the sun few times to reduce moisture content. Clean and shiny bright color of dry seed indicated high quality of the seeds. This was laborious but is a simple technique of quality seed saving in small scale. Such healthy seeds with moisture content less than 12% will retain viability and seed strength required for healthy seedling development without seed treatment.

Segregating breeding lines of seed color mutants were valuable genetic resources showing diversity for color, seed size, pod and total seed yield. The diversities for traits like nutritional content, disease resistance, insect resistance, plant growth patterns were not studied. Seed coat cracking was seen but was not seriously considered in this study. However, promising lines with desirable agronomic traits are marked from the list of each population with the intention of advancing generation and further testing in order to develop varieties . Selected lines within a population with similar seed color could be bulked and planted for generation advance and practice bulk selection. These lines are valuable genetic resources with multiple use potential in the future from disease resistance to human health and nutrition. Brown and black seed edamame are the creation at the Sharing Farm. It is important to locate certain research and academic organization that can conserve the genetic diversity by entering into gene pool of edamame.

Appendix I. Data on segregating M_2 seed color mutants of SPS- UBC-Isophenic edamame breeding lines grown at Sharing Farm during 2015.

Entry Plant	Plht (cm)	Branch (#)	Nodes (#)	Pod Yld (g)	Pods (#)	1-bean (%)	2-bean (%)	3-bean (%)	Seed Yld (g)	SW/100 (g)	Color code	Crack rating
UBC1501	**45**	**2**	**11**	**92**	**60**	**27**	**53**	**20**	**43**	**50**	**2**	**0**
UBC1502	**40**	**2**	**11**	**75**	**54**	**28**	**43**	**29**	**40**	**45**	**2**	**1**
UBC1503	**38**	**3**	**11**	**66**	**49**	**35**	**41**	**24**	**36**	**48**	**2**	**0**
UBC1504	**48**	**3**	**13**	**81**	**45**	**15**	**58**	**27**	**34**	**45**	**2**	**0**
UBC1505	**45**	**3**	**13**	**85**	**69**	**19**	**48**	**33**	**50**	**44**	**2**	**1**
UBC1506	**52**	**3**	**12**	**108**	**103**	**29**	**50**	**21**	**63**	**45**	**2**	**0**
UBC1507	**42**	**2**	**12**	**77**	**37**	**29**	**41**	**30**	**34**	**45**	**2**	**0**
UBC1508	**40**	**2**	**11**	**53**	**44**	**18**	**57**	**25**	**30**	**45**	**2**	**0**
UBC1509	42	2	8	36	26	35	50	15	19	45	2	0
UBC1510	**46**	**3**	**15**	**176**	**95**	**32**	**46**	**22**	**63**	**50**	**2**	**0**
UBC1511	**40**	**3**	**12**	**136**	**79**	**29**	**51**	**20**	**62**	**46**	**2**	**0**
UBC1512	**50**	**3**	**12**	**166**	**105**	**29**	**42**	**29**	**63**	**50**	**2**	**0**
UBC1513	**25**	**2**	**8**	**87**	**52**	**17**	**52**	**31**	**41**	**46**	**2**	**1**
UBC1514	23	1	7	33	20	15	50	35	12	42	2	1
UBC1515	24	2	9	31	15	34	33	33	13	52	2	0
UBC1516	**17**	**2**	**8**	**45**	**27**	**18**	**63**	**19**	**27**	**52**	**2**	**0**
UBC1517	22	1	8	36	23	31	65	4	11	50	2	1
UBC1518	22	1	7	26	16	18	44	38	15	50	2	1
UBC1519	21	1	7	28	19	15	74	11	16	43	2	0
UBC1520	34	1	8	41	33	3	67	30	26	36	2	0
UBC1521	24	1	7	14	19	47	32	21	7	26	2	1
UBC1522	25	0	7	19	18	45	50	5	10	33	2	0
UBC1523	34	0	7	13	12	17	50	33	5	25	2	0
UBC1524	20	1	7	18	17	23	59	18	10	27	2	0
UBC1525	35	0	7	11	12	8	67	25	5	20	2	0

UBC1526	36	0	8	14	13	8	54	38	7	26	2	0
UBC1527	40	2	11	11	8	26	37	37	5	30	2	1
UBC1528	35	3	8	21	10	40	30	30	10	48	2	1
UBC1529	20	1	8	13	15	14	80	8	5	20	2	2
Mean Range	**33.9 17-52**	**1.7 0-3**	**9.4 7-15**	**55.0 11-176**	**37.7 8-105**	**24.2 3-47**	**51.3 30-80**	**24.5 4-38**	**26.7 5-63**	**40.8 20-52**	**Brown 29/29**	**0=20;1=8 2=1**

Mutant obtained from UBC grown population in 2013 (M1 plant). Entry code, UBC 11501= University of British Columbia -2015 plant 01 to 29. Promising entries are marked with bold face. Seed coat color rating: 1= Yellow; 2=Brown; 3=Black; 4=Beize

Appendix II. Data on segregating M_3 seed color mutants of SPS- BL-3 edamame population grown at Sharing Farm during 2015.

Entry plant	Plht (cm)	Nodes (#)	Branch (#)	Pod Yld (%)	Pod (#)	1-bean (%)	2-bean (%)	3-bean (%)	Seed Yld (g)	SW/100 (g)	Color code	Crack rating
MBL31501	**14**	**7**	**1**	**30**	**12**	**25**	**42**	**33**	**13**	**50**	**2**	**0**
MBL31502	30	9	2	26	13	23	54	23	8	40	2	1
MBL31503	**19**	**7**	**1**	**32**	**22**	**28**	**45**	**27**	**16**	**40**	**4**	**1**
MBL31504	22	10	2	75	33	25	36	39	18	40	4	0
MBL31505	10	6	1	13	6	17	83	0	5	45	2	0
MBL31506	17	9	2	18	7	15	71	14	5	40	1	0
MBL31507	15	7	1	37	15	13	47	40	13	50	1	2
MBL31508	**15**	**9**	**0**	**14**	**9**	**22**	**56**	**22**	**6**	**45**	**1**	**2**
MBL31509	28	8	2	22	14	15	71	14	11	30	4	1
MBL31510	15	6	1	9	5	20	60	20	11	30	1	2
MBL31511	34	11	3	18	10	30	30	40	7	40	2	1
MBL31512	27	12	1	6	11	28	36	36	4	15	3	0
MBL31513	**36**	**14**	**1**	**27**	**23**	**21**	**57**	**22**	**15**	**40**	**1**	**0**
MBL31514	32	10	2	21	23	26	57	17	11	25	2	1
MBL31515	25	7	0	7	6	0	50	50	2	15	1	0
Mean **Range**	**22.6** **10-36**	**8.8** **6-14**	**1.3** **0-3**	**23.7** **6-75**	**13.9** **5-33**	**20.5** **0-30**	**53.0** **30-83**	**26.4** **0-50**	**9.6** **2-18**	**36.3** **15-50**	**1=6;2=5** **3=1;4=3**	**0=7;1=5;** **2=3**

Data on segregating M3 plants originated from SPS- BL 3 population (seed color mutation on 2011, M1 plant grown on 2012 and M2 plants grown during 2015) and M3 grown at Sharing Farm during 2015. Entry code MBL31501= Mutant breeding line 3 2015 plant 01-15. Promising entries are marked with bold face. Seed coat color rating: 1= Yellow; 2=Brown; 3=Black; 4=Beize

Appendix III. Data on segregating M_3 seed color mutants of SPS- BL-1 edamame population grown at Sharing Farm during 2015.

Entry plant	Plht (cm)	Nodes (#)	Branch (#)	Pods (#)	1-bean (%)	2-bean (%)	3-bean (%)	Seed Yld (g)	SW/100 (g)	Color	Crack
MBL11501	**44**	**10**	**3**	**39**	**23**	**62**	**15**	**34**	**52**	**3**	**0**
MBL11502	40	10	3	8	25	50	20	5	40	3	1
MBL11503	38	8	3	37	27	46	27	23	41	3	1
MBL11504	**33**	**9**	**4**	**58**	**40**	**45**	**15**	**48**	**51**	**3**	**0**
MBL11505	34	9	2	17	59	29	12	11	46	3	0
MBL11506	**50**	**10**	**3**	**75**	**34**	**41**	**25**	**56**	**48**	**2**	**0**
MBL11507	**50**	**13**	**4**	**92**	**34**	**51**	**15**	**70**	**51**	**3**	**0**
MBL11508	44	10	3	50	44	48	8	29	37	3	0
MBL11509	40	8	2	29	28	48	24	24	46	3	0
MBL11510	**34**	**8**	**2**	**32**	**44**	**47**	**9**	**23**	**48**	**3**	**0**
MBL11511	42	7	2	40	37	40	23	23	43	3	0
MBL11512	**43**	**9**	**2**	**34**	**50**	**29**	**21**	**25**	**51**	**2**	**0**
MBL11513	54	13	2	53	38	43	19	21	26	2	1
MBL11514	23	9	2	26	15	58	27	15	38	3	0
MBL11515	**30**	**8**	**4**	**56**	**22**	**53**	**25**	**41**	**47**	**3**	**1**
MBL11516	22	7	2	40	0	48	52	27	32	3	1
MBL11517	**44**	**11**	**4**	**71**	**32**	**37**	**31**	**20**	**25**	**3**	**1**
MBL11518	46	11	2	25	14	64	20	19	44	3	0
MBL11519	**38**	**8**	**4**	**44**	**29**	**41**	**30**	**31**	**42**	**3**	**0**
MBL11520	**30**	**7**	**1**	**25**	**12**	**52**	**36**	**25**	**42**	**3**	**1**
MBL11521	40	9	1	29	28	65	7	18	38	3	0
MBL11522	**34**	**9**	**1**	**21**	**19**	**38**	**43**	**19**	**50**	**3**	**0**
MBL11523	42	8	1	12	17	50	33	11	25	1	1
MBL11524	22	8	0	10	30	40	30	5	25	3	0
MBL11525	40	8	1	14	35	29	36	5	21	3	0

MBL11526	40	9	4	31	32	55	13	11	25	2	1
MBL11527	30	8	1	19	47	32	21	5	22	2	1
MBL11528	28	8	3	10	60	40	0	5	50	3	0
MBL11529	30	7	3	15	30	67	13	11	43	3	0
MBL11530	36	10	3	13	31	62	7	7	33	2	1
MBL11531	40	8	3	20	15	60	35	12	45	3	1
MBL11532	25	7	2	13	23	62	15	7	32	3	1
MBL11533	38	10	2	11	27	45	27	4	30	2	1
MBL11534	34	8	1	6	33	50	17	5	50	2	0
MBL11535	24	8	3	16	12	50	38	7	30	3	1
MBL11536	16	6	2	11	31	50	19	12	48	3	1
MBL11537	16	7	3	29	32	50	18	15	46	3	1
MBL11538	15	8	2	22	18	64	28	13	37	3	1
MBL11539	9	7	2	27	19	81	1	17	47	3	0
MBL11540	11	7	2	11	46	36	18	5	30	3	0
MBL11541	12	5	1	11	9	73	18	6	32	3	0
MBL11542	22	7	1	11	28	45	27	9	41	3	0
MBL11543	30	8	1	29	34	59	7	10	26	2	0
MBL11544	27	9	1	11	9	64	27	7	35	3	0
MBL11545	20	5	0	7	29	57	14	6	50	3	0
MBL11546	45	8	2	16	6	31	63	11	28	3	0
MBL11547	42	8	2	30	53	20	27	15	38	3	0
MBL11548	30	8	1	19	11	63	26	12	38	3	0
MBL11549	44	7	2	16	50	37	13	10	36	3	0
MBL11550	35	7	2	21	29	57	14	13	35	3	0
MBL11551	40	10	2	44	48	36	16	23	32	3	0
MBL11552	48	8	2	42	43	43	14	23	32	3	0
MBL11553	48	10	2	38	31	37	32	22	35	3	0
MBL11554	40	11	4	62	33	47	26	43	43	3	0

MBL11555	**27**	**11**	**4**	**83**	**22**	**44**	**34**	**33**	**22**	**3**	**0**
MBL11556	46	10	2	31	51	43	6	21	45	2	0
MBL11557	46	10	3	43	46	40	14	29	45	3	0
MBL11558	28	9	2	44	32	54	14	21	30	3	0
MBL11559	40	9	2	40	28	45	27	21	31	3	0
MBL11560	22	8	2	25	24	40	36	16	36	1	0
MBL11561	15	5	1	24	17	54	29	16	25	3	0
MBL11562	44	10	3	32	32	40	28	14	25	3	0
MBL11563	22	6	1	21	29	38	33	15	35	3	0
MBL11564	48	10	2	32	19	53	28	18	26	3	0
MBL11565	**52**	**11**	**2**	**31**	**45**	**45**	**10**	**19**	**44**	**2**	**0**
MBL11566	50	10	3	50	34	56	10	16	25	3	0
MBL11567	28	8	1	26	50	27	23	12	30	3	0
MBL11568	34	9	2	32	38	34	28	13	25	3	0
MBL11569	33	8	1	24	33	42	25	15	35	3	0
MBL11570	42	9	2	23	35	39	26	14	31	3	0
MBL11571	55	9	0	15	27	53	20	8	30	1	0
MBL11572	28	11	2	12	9	58	33	7	20	3	0
MBL11573	41	8	1	20	25	65	10	11	26	2	0
MBL11574	26	8	1	14	19	21	50	7	23	2	0
MBL11575	16	6	1	16	12	75	13	12	35	3	0
MBL11576	23	6	1	20	25	65	10	9	22	3	0
MBL11577	23	7	1	19	21	42	37	8	20	3	0
MBL11578	38	8	1	18	39	50	11	7	21	3	0
MBL11579	18	7	2	13	31	38	31	7	25	3	0
MBL11580	30	9	2	12	67	33	0	7	35	3	0
Mean Range	**33.8 9-55**	**8.4 5-13**	**2.0 0-4**	**28.3 6-02**	**30.1 0-67**	**47.7 20-81**	**22.2 0-63**	**16.8 4-70**	**35.5 20-5**	**1=3;2=13 3=64**	**0=62 1=18**

Data on segregating M3 plants originated from SPS- BL 1 population (seed color mutation on 2011, M1 plant grown on 2012 and M2 plants grown during 2013) and M3 grown at Sharing Farm during 2015. Entry code MBL11501= Mutant breeding line -1-2015-plant 01 to 80. Promising entries are marked with bold face. Seed coat color rating: 1= Yellow; 2=Brown; 3=Black; 4=Beize

Appendix IV. Data on segregating M_3 seed color mutants of SPS- Isophenic -BL-2 edamame population grown at Sharing Farm during 2015.

Entry plant	Plht (cm)	Node (#)	Branch (#)	Pods (#)	1-bean (%)	2-bean (%)	3-bean (%)	Seed Yld (g)	SW/100 (g)	Color code	Crack Rating
MBL-I-15-01	**80**	**16**	**2**	**37**	**16**	**60**	**24**	**29**	**47**	**2**	**1**
MBL-I-15-02	30	9	2	10	10	40	50	6	30	2	1
MBL-I-15-03	76	14	2	18	44	39	17	11	40	2	1
MBL-I-15-04	**80**	**16**	**2**	**75**	**3**	**64**	**33**	**49**	**40**	2	1
MBL-I-15-05	**105**	**16**	**2**	**84**	**31**	**49**	**20**	**47**	**42**	2	0
MBL-I-15-06	**80**	**14**	**1**	**72**	**37**	**42**	**21**	**42**	**43**	2	0
MBL-I-15-07	**102**	**17**	**1**	**46**	**26**	**41**	**33**	**28**	**36**	2	0
MBL-I-15-08	90	14	1	37	51	35	14	20	48	2	0
MBL-I-15-09	85	15	2	19	46	33	21	11	35	2	0
MBL-I-15-10	**75**	**16**	**1**	**63**	**23**	**55**	**22**	**31**	**42**	**2**	**1**
MBL-I-15-11	**62**	**14**	**1**	**79**	**33**	**43**	**24**	**43**	**36**	**2**	**0**
MBL-I-15-12	**67**	**14**	**2**	**75**	**29**	**48**	**23**	**38**	**35**	**2**	**0**
MBL-I-15-13	54	16	3	42	26	59	15	18	36	2	1
MBL-I-15-14	**70**	**13**	**2**	**39**	**24**	**45**	**31**	**30**	**45**	**2**	**1**
MBL-I-15-15	60	16	1	24	70	17	13	13	36	2	0
MBL-I-15-16	56	12	1	14	65	35	0	9	41	2	0
MBL-I-15-17	60	10	0	13	23	54	23	11	41	2	0
MBL-I-15-18	75	14	0	19	21	42	37	13	40	2	0
MBL-I-15-19	68	14	1	19	42	37	21	13	35	2	1
MBL-I-15-20	54	11	1	16	24	47	29	10	26	2	2
MBL-I-15-21	65	11	0	28	25	43	32	15	25	2	0
MBL-I-15-22	55	10	0	29	13	66	21	14	32	2	0

MBL-I-15-23	65	10	0	13	50	43	7	9	35	2	0
MBL-I-15-24	**32**	**9**	**3**	**33**	**36**	**62**	**2**	**22**	**48**	**2**	**1**
MBL-I-15-25	44	10	1	16	25	69	6	12	40	2	0
MBL-I-15-26	40	10	2	20	10	60	30	13	35	2	1
MBL-I-15-27	**56**	**14**	**1**	**52**	**52**	**38**	**10**	**24**	**36**	**2**	**0**
MBL-I-15-28	44	11	2	29	31	52	17	20	45	2	0
MBL-I-15-29	24	8	2	17	24	47	29	11	35	2	1
MBL-I-15-30	18	7	0	16	34	31	25	14	47	2	0
MBL-I-15-31	20	6	1	12	17	58	25	12	47	2	0
MBL-I-15-32	20	5	1	21	29	62	9	10	30	2	2
MBL-I-15-33	54	13	1	21	24	38	38	9	20	2	1
MBL-I-15 34	13	5	2	10	30	40	30	8	36	2	1
MBL-I-15-35	17	7	2	11	8	73	19	8	35	2	0
MBL-I-15-36	46	9	1	16	12	63	25	6	20	2	0
MBL-I-15-37	48	9	1	15	21	33	46	8	26	2	0
MBL-i-15 -38	52	9	0	12	25	67	8	6	25	2	0
MBL-I-15-39	21	7	0	9	23	44	33	7	35	2	0
MBL-I-15-40	30	8	1	9	45	44	11	6	35	2	1
MBL-I-15-41	25	8	0	13	61	31	8	9	40	2	0
MBL-I-15-42	23	7	1	12	25	42	33	5	30	2	0
MBL-I-15-43	30	7	1	17	53	47	0	10	45	2	0
MBL-I-15-44	25	8	1	10	70	30	0	9	50	2	0
MBL-I-15-45	48	10	1	11	37	36	27	7	21	2	0
MBL-I-15-46	30	6	1	12	25	75	0	10	36	2	0
MBL-I-15-47	27	8	1	14	36	57	7	7	20	2	0
MBL-I-15-48	24	8	1	8	37	63	0	7	40	2	0
MBL-I-15-49	55	12	1	11	46	27	27	5	25	2	0
MBL-I-15-50	31	8	0	8	24	63	13	5	20	2	0
MBL-I-15-51	**60**	**16**	**3**	**68**	**36**	**49**	**15**	**33**	**28**	**2**	**0**

MBL-I-15-52	55	12	2	17	41	53	6	10	28	2	0
MBL-I-15-53	65	12	2	16	27	58	15	10	25	2	0
MBL-I-15-54	60	14	3	43	51	33	16	18	27	2	0
MBL-I-15-55	48	13	3	38	34	61	5	21	33	2	0
MBL-I-15-56	35	10	2	30	10	57	33	16	22	2	0
MBL-I-15-57	30	8	1	25	32	36	32	15	32	2	0
MBL-I-15-58	40	11	0	15	33	20	47	8	20	2	0
MBL-I-15-59	20	7	1	18	39	50	11	12	35	2	0
MBL-I-15-60	21	8	2	39	28	62	10	23	37	2	0
MBL-I-15-61	19	7	1	10	30	40	30	9	40	2	0
MBL-I-15-62	20	8	2	14	15	64	21	8	23	2	0
MBL-I-15-63	33	8	0	10	40	50	10	6	20	2	0
MBL-I-15-64	32	8	0	13	39	46	15	7	23	2	2
MBL-I-15-65	14	5	0	6	0	50	50	6	33	2	0
MBL-I-15-66	30	6	1	12	33	42	25	6	21	2	0
MBL-I-15-67	26	7	0	9	33	56	11	5	20	2	0
MBL-I-15 -68	23	5	0	8	17	75	13	6	22	2	0
MBL-I-15-69	35	9	0	6	33	50	17	4	20	2	0
Mean **Range**	**46.0** **13-105**	**10.3** **5-17**	**1.1** **0-3**	**24.6** **6-84**	**31.3** **0-70**	**48.4** **17-75**	**20.1** **0-50**	**14.5** **4-49**	**33.2** **20-50**	**Brown** **69/69**	**0=53;1=13** **2=3**

Data on segregating M3 plants originated from SPS- Isophenic population (seed color mutation on 2011, M1 plant grown on 2012 and M2 plants grown during 2013) and M3 grown at Sharing Farm during 2015. Entries were coded as MBL-I-15-01 to MBL-I-15-69 (mutant breeding lines Isophenic 15-01 to 69). Promising entries are marked with bold face. Seed coat color rating: 1= Yellow; 2=Brown; 3=Black; 4=Beize.

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