THE DEVELOPMENT OF A NEW HYBRID SWEET SHOOT VARIETY 'WRINKIE'

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Abstract

The sweet shoot, a common vegetable crop in Sarawak, has been given little attention as it is relatively an easy crop to grow. This scenario was taken over by events due to the outbreak of the bacterial stem blight disease (BSBD) incidence in the late '90s. BSBD which is a fatal disease caused by *Xanthomonas campestris* bacteria has wiped out a number of large commercial sweet shoot farms in Miri, Samarahan and Kuching Divisions. Since we had not been able to control the disease by agrochemical means, a breeding programme was initiated in 1999 as an alternative approach to overcome the disease. The main breeding objective was to breed for a resistant hybrid variety with high yield and quality shoots. The resultant elite variety or clone would then be recommended to farmers who wish to grow the crop, especially for the disease-prevailing areas.

Under the crop improvement programme initiated by the vegetable research section at Semongok Agricultural Research Centre (ARC) in 1999, some selected accessions of sweet shoots were crossed. The progenies, First filial generation hybrids (F1 hybrids), were assessed on disease resistance, shoot yield and other relevant agronomic parameters. Field trials and on-farm verifications have proven that a selected new F1 hybrid named 'Wrinkie' has several outstanding traits, such as good yield, good eating quality shoots and good bacterial stem blight resistance. This recommended variety is now being made available to farmers for planting. The 'Wrinkie' variety has very crinkle dark green leaves which are sweet and very crispy. Market testing sales by farmers have shown its acceptance by consumers, especially for 'fast food' and 'Lui cha' food vendors.

INTRODUCTION

The sweet shoot or star gooseberry, locally known as 'changkok manis' and also as 'cekur manis' in Peninsular Malaysia, is botanically identified as *Sauropus androgynus* (L.) Merrill. It is a member of the *Phyllanthaceae* family (previously *Euphorbiaceae*). Its exact origin is unknown but it has been growing from India to Sri Lanka and Southern China and down to Indochina and throughout the South East Asia, mainly in cultivated areas as well as in the wild (Van de Bergh, 1994). The species have really been neglected, despite its valuable economic, medicinal and nutritional contributions. PROSEA even recorded that no germplasm collection and breeding are in progress. It claimed that it (sweet shoot) has not received adequate research attention and very little new information has been generated during the last 50 years! (*Ibid*). The present work at ARC, perhaps could help in a small way towards the breeding and research of this neglected plant species.

Sweet shoot plants are erect, glabrous, perennial and monoecious. Stems and branches are cylindrical tapering and slender. The inflorescence consist both male and female flowers on the same leaf stalk. The fruits are 6-angular capsules. The plants may be propagated either by seeds or stem cuttings. Its suitability for vegetative propagation is an advantage for cloning of any desired variety.

The sweet shoots are a common but welcome vegetable at home and in the food outlets in the towns and cities throughout Sarawak. The vegetable is very nutritious, containing very high protein, Calcium and Vitamin A. Some people have pains in limbs after heavy consumption (*Ibid*). The production statistics from 2002 to 2005 indicated a slight decline for two years and regained again in total area grown in the

State of Sarawak (Table 1) (Anon, 2004, 2005, 2006, 2007). As a leafy vegetable, it may be prepared in a number of ways, including soups, quick oil fried and in mixed servings with other vegetables. This vegetable is specially sought for in the preparation of 'Hakka Lui cha' which is an herbal soup with mixed vegetables served normally with rice.

Table 1: Major sweet shoot growing areas in Sarawak (CHE*)

Division	Year			
	2002	2003	2004	2005
Kuching	31.2	18.3	21.4	30.2
Samarahan	21.2	7.5	9.9	10.4
Sri Aman	2.6	7.5	11.4	16.0
Betong	10.4	0.5	6.7	7.3
Sibu	19.1	9.9	17.9	18.2
Sarikei	23.3	12.5	15.0	14.9
Bintulu	1.4	1.8	3.0	5.3
Miri	9.4	4.9	5.4	5.4
Kapit	4.0	3.4	0.1	10.2
State Total	129.8	69.5	93.8	121.8

* Crop hectareage equivalent.

There has been little attention given to this crop until the occurrence of BSBD incidence in the late '90s. This fatal disease caused by *Xanthomonas campestris* bacteria has wiped out a number of large commercial farms in Miri, Samarahan and Kuching Divisions. Attempts made to control the disease by means of spraying with agrochemicals and antibiotics in 1991 in a farmer's infected field at Kampong Patung Road, Samarahan, were without success (Teo, 1991). Since we were not able to control the BSBD by agrochemical means, a breeding programme was initiated in 1999 as an alternative approach to disease control. The breeding objective was to have crosses from the available germplasm accessions in order to select for a BSBD resistant variety with high yield and quality shoots. The resultant elite variety or clone would then be recommended to farmers who wish to grow the crop, especially for the disease-prevailing areas.

GERMPLASM COLLECTIONS AND SELECTION

The collections of germplasm on sweet shoot in the State were first carried out in 1995. In September of the same year, 14 accessions were field planted in single rows of 20 - 30 points, with plants spaced at 30 cm apart, for observation at ARC. Two kg/bed of chicken dung at once a month and 400 g/bed of NPKMg+TE granulated compound fertiliser at twice a month were applied to these plants throughout the experimental period. Visual scoring of the disease incidence was made in September 1996 and presented in Table 2 (Teo & Chai, 1996). The Table indicates that with respect to BSBD, 'Cekur manis' (CM) accessions 'CM111', 'CM112' and 'CM113' have very high resistance while 'CM101' has a high tolerance. 'CM105', 'CM106', 'CM107' and 'CM108' are highly susceptible.

In 1997, eight 'cekur manis' accessions in the ARC germplasm were selected for evaluations on yield and other agronomic traits at ARC, with the view of getting elite accessions for starting the breeding programme (Anon. 1998). The crop was planted in October, 1997 and harvested from 13/01/1998 to 30/12/1998. The fertiliser regime used was the same as the earlier observation plot. Table 3 shows that accessions 'CM 100', 'CM 101', 'CM 111', 'CM 112', 'CM 113' and 'CM 201' had outstanding fresh shoot yields. But only 'CM 111' and 'CM 112' were able to fully survive the bacterial stem blight disease at the end of the trial.

Table 2: BSBD incidence in the germplasm evaluation trial

Accession No. (CM)	Plants infected (%)
111	0.0
112	0.0
113	1.0
101	19.2
100	37.5
104	56.3
109	68.4
110	71.4
102	81.8
103	83.3
105	100.0
106	100.0
107	100.0
108	100.0

Table 3: Yield and observed traits of some 'Cekur manis' accessions

Accession No. (CM)	Shoot yield (mt/ha/year)*	Remarks
100	31.4 b	Plant medium vigour, medium sized leaves, small stems, good quality leaves, medium BSBD susceptibility.
101	39.7 ab	Plant medium vigour, thin medium leaves, small stems, poor quality leaves, medium BSBD susceptibility.
111	42.8 a	Plant vigorous, thin large leaves, small stems, may be native plant, poor quality leaves, excellent BSBD resistance.
112	34.8 ab	Plant vigorous, thin large leaves, small stems, may be a native plant, poor quality leaves, excellent BSBD resistance.
113	36.8 ab	Plant vigorous, thin large leaves, small stems, native plant, poor quality leaves, good BSBD tolerance.
114	18.9 c	Plant vigorous, thick medium sized leaves, large stems, may be not a native plant, good quality leaves, very susceptible to BSBD.
115	15.5 c	Plant vigorous, thick medium sized leaves, large stems, may be not a native plant, good quality leaves, very susceptible to BSBD.
201	34.8 ab	Plant vigorous, thick large waxy leaves, large stems, may be not a native plant, good quality leaves, very susceptible to BSBD.
Mean	31.8	
SED	4.02	
CV (%)	17.8	

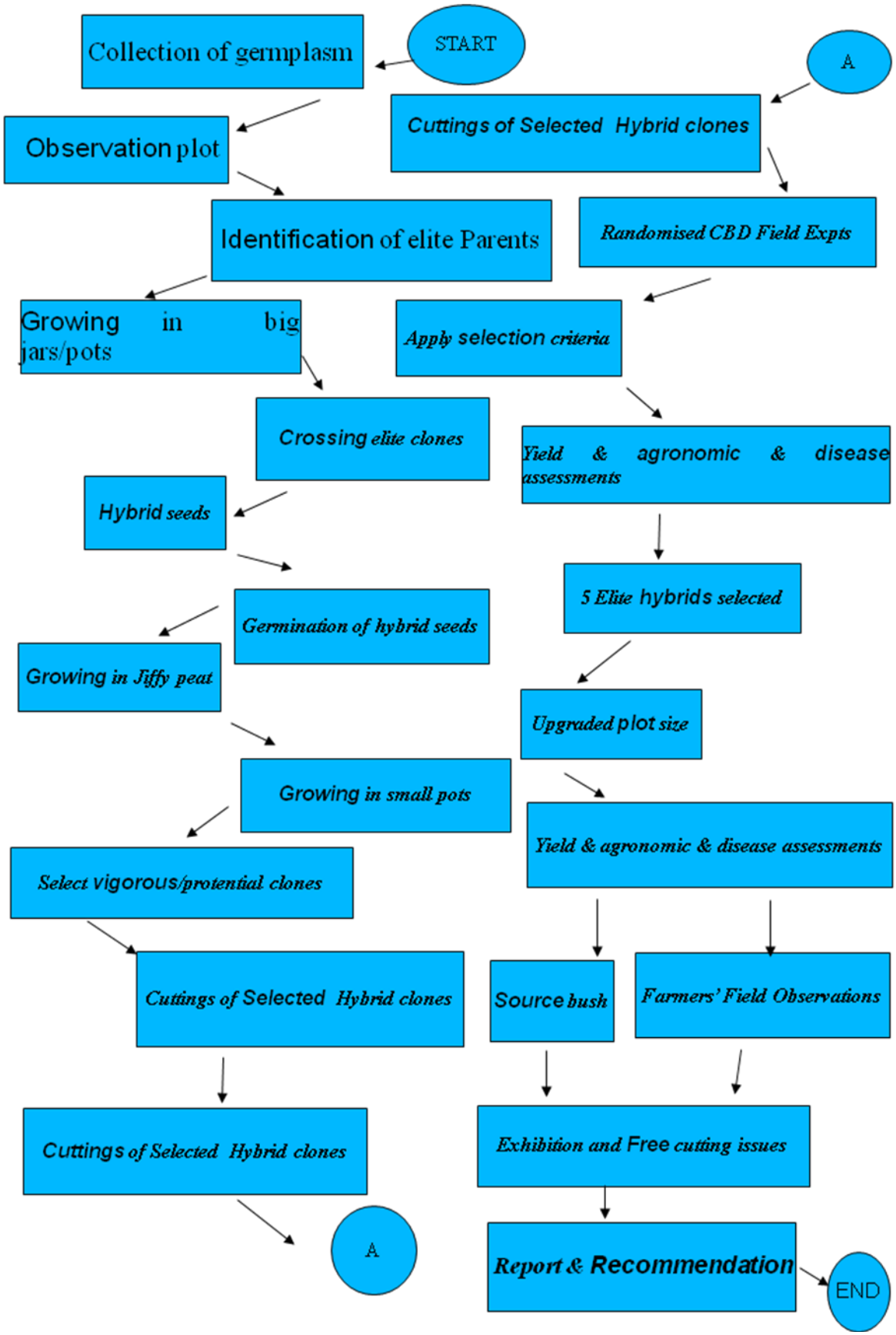
*Figures within a column with the same letter are not significantly different at P=0.05 by Duncan Multiple Range Test

SWEET SHOOT BREEDING PROGRAMME

Breeding objectives and selection for desired traits

A crop improvement programme on the 'cekur manis' was initiated in 1999 to provide an alternative means of BSBD control. The results of Tables 2 and 3 indicated that there were no one accession which had both quality yield and very good BSBD resistance. The objective of the programme was therefore primarily to produce one or more 'cekur manis' hybrid varieties not only with the resistant/tolerant genes but also possessing other desirable traits such as good shoot yield, good eating quality shoots, attractive appearance and good physical attributes. The variety must also be easily propagated and cultivated.

Basing on the set selection criteria, all the 'Cekur manis' accessions in the germplasm that possessed the traits of interest were chosen to be the parents for crossing. The flow chart for the breeding process is as depicted in Chart 1.


START
Collection of germplasm
Observation plot
Identification of elite Parents
Growing in big jars/pots
Crossing elite clones
Hybrid seeds
Germination of hybrid seeds
Growing in Jiffy peat
Growing in small pots
Select vigorous/protential clones
Cuttings of Selected Hybrid clones
Cuttings of Selected Hybrid clones
A
A
Cuttings of Selected Hybrid clones
Randomised CBD Field Expts
Apply selection criteria
Yield & agronomic & disease assessments
5 Elite hybrids selected
Upgraded plot size
Yield & agronomic & disease assessments
Source bush
Farmers' Field Observations
Exhibition and Free cutting issues
Report & Recommendation
END

Selection of Parents, crossings, F1 hybrids culture and evaluations

Five crosses (combinations), as indicated in Table 4, were made from the selected elite accessions of Tables 2 and 3 based on the set criteria discussed earlier. The female parents were 'CM 111' and 'CM 112' which were known to be of high resistance to BSBD. The male parents were good eating accessions but having medium to poor resistance to the disease. In addition, 'CM211' which was known as a 'sweet type' was also included. The F1 hybrid seeds were extracted from the mature pods and germinated in 'Jiffy peat' pots. Twenty-two (22) F1 hybrid seedlings were finally transferred from these Jiffy pots and established in small flower pots. Some of the seeds were not viable while some others germinated but were too weak to make it to the seedling stages. These hybrid seedlings were successfully cultured to 1- metre in height and then pruned to get cuttings for planting to large egg jars. The first field observation plot was established from the stem cuttings derived from these egg-jar grown hybrid F1 seedlings. The plot also served as a source bush.

Table 4: Parentage of sweet shoot hybrids

Cross	Parent (CM)		No. of progeny(Plant Number)	Date crossed	Date hybrid seed planted
(Progeny)	Male	Female			
201 x 111	201	111	5 (P1-5)	02/09/99	11/10/99
201 x 112	201	112	13 (P1-13)	18/09/99	28/10/99
100 x 112	100	112	2 (P1- 2)	04/11/99	21/12/99
100 x 111	100	111	2 (P1-2)	17/11/99	21/12/99
201 x 211	201	211	8 (P1-8)	18/09/99	28/10/99

F1 hybrids observation at ARC

A field plot with the cuttings from the 22 egg jar grown hybrid seedlings was successfully established at ARC for making preliminary field observations and to serve as a planting material source bush area. This was the first hybrid progeny field screening on survival in an area with BSBD history. Hybrid vigour was strongly expressed by the progeny of 'CM 201', having large leaves and big stems while some other crosses appeared weak and having problems with field establishment (Anon. 2000). From visual appraisals, survival rates and tasting of cooked shoots, only 12 hybrid progenies were picked up for a second field assessment.

Hybrid progeny and accessions trial

Twelve selected hybrid progenies, two susceptible ('CM116' and 'CM117') and two highly BSBD tolerant ('CM111' and 'CM112') accessions were compared in a randomised complete block design experiment at ARC. The objective was to compare the new hybrid clones against the available accessions for the desired traits under examination, especially on yield and most importantly the resistance to BSBD.

In October 2001, the selected 'cekur manis' entries were field planted in raised beds measuring 1.2 m by 7.2 m. Forty-eight (48) points of each clone were grown in each treatment plot at a spacing of 30 cm by 60 cm in double rows. Each treatment clone was replicated three times. Two kg/bed of chicken dung at once a month and 400 g/bed of 12:12:17:2+TE, NPKMg+TE, granulated compound fertiliser at twice a month were applied to the plants throughout the experimental period. Weekly sprays of 'Mitac' and 'Biogreen' were carried out after each harvest to control mites and thrips. The plants were harvested six times in the year. Yield, other agronomic parameters and disease incidence were recorded. The ease of propagation and field establishment of the hybrids were also noted.

Two years of harvests were carried out. The trial was terminated when one treatment had all the plants in the replicates died as a result of BSBD infections. The characteristic yellow stem streaks and chlorotic leaf symptoms of the disease were evident and the causative agent was pathologically confirmed in the laboratory by Teo (personal communications, 2002). The results presented in Table 5 show large significant differences in the shoot yields and BSBD survivals. There were at least five hybrids with outstanding shoot yield, tolerance to BSBD and having good physical attributes such as good appearance and strong stems.

Table 5: Performance of 'cekur manis' F1 hybrids and accessions at ARC*

Selected accession or hybrid CM (Given name)	Shoot yield (mt/ha/year)		Number of surviving plants (%) on 31st December	
	2002	2003	2002	2003
111	11.89 bcd	24.32 abc	92.3 ab	88.3 abc
112	11.83 bcd	19.24 bcd	92.7 ab	91.0 ab
116	9.32 de	2.42 hi	15.0 f	0.7 h
117	20.70 a	4.54 ghi	39.7 e	2.7 h
201-112/P1**	7.74 de	16.29 cdef	68.7 bcd	61.7 def
201-112/P2	11.04 cde	12.26 defg	81.3 abcd	39.7 fg
201-112/P4 ('Cutie')	9.80 de	13.37 def	84.3 abcd	67.7 bcde
201-112/P7	2.21 f	0.00 i	1.3 f	0.0 h
201-112/P10	6.75 e	0.53 i	10.3 f	2.7 h
201-111/P1	8.36 de	8.07 fghi	66.7 cd	46.7 efg
201-111/P2	7.93 de	9.02 efgh	60.3 de	35.3 g
201-111/P3	8.09 de	17.30 cde	87.3 abc	78.0 abcd
100-111/P1 ('Wrinkie')	**16.78 ab**	**27.99 a**	**96.0 a**	**94.0 a**
100-111/P2 ('Greenie')	14.99 bc	22.02 abc	84.0 abcd	66.7 cde
100-112/P1 ('Waxie')	10.32 cde	16.73 cde	63.3 cde	43.0 fg
100-112/P2 ('Eggie')	16.32 ab	26.52 ab	98.0 a	86.0 abc
Mean	10.8	13.8	65.1	50.2
SED	2.05	3.58	10.43	9.8
CV (%)	23.1	31.8	19.6	23.9

* Figures within a column with the same letter are not significantly different at P=0.05 by Duncan Multiple Range Test

** P- Plant number derived from germinated seeds of pods from the same parental cross.

Upscale field performance of selected hybrids

Based on the two year trial results of the progeny and selected accessions (Table 5) on the set parameters such as shoot yield, survival rate from the BSBD and physical attributes, five hybrids were chosen and upscale to larger plots in 2004 to assess for the third time the progeny performance at ARC.

Each hybrid was planted on 24 raised beds of 1.2 m by 6 m (172.8 m^2; 0.04 ac) in single rows. The beds were covered with 80% black shade net to reduce weeding and to provide mulch. The same fertiliser rates as the progeny trial described earlier were used for this trial. Annual yield is the total shoot yield of harvests of each treatment hybrid plot for the year recorded. Table 6 shows that the hybrid clone 'Wrinkie' has the best averaged shoot yield and the best plant survival at the completion of the trial. It could be concluded that the F1 hybrid 'Wrinkie' was the best of the hybrid progeny derived from the breeding programme based on these two important parameters. Table 7 has the foliage traits of the five selected clones in the upscale observation plot at ARC.

QUALITY ASSESSMENTS

Both yield (quantity) and quality traits are important to a crop. To farmers both are relevant while the consumers want the quality more. Table 8 shows the agronomic traits of 'Wrinkie'.

Foliage nutritive values

The nutritional contents on dry weight basis of 'Wrinkie' are: 33.46 % protein; 27.20 % carbohydrates; 24.38% Fats; Acidity 0.69%; Crude Fibre 5.34% (medium amongst the 5 clones); Ash 9.62 %; Phosphorus 0.76 %; Potassium 3.10 %; Calcium 0.72 %; Sulphur 0.31 %; Magnesium 0.41 %; Iron 107 ppm ; Manganese 45 ppm; Copper 9 ppm; Zinc 57 ppm and Boron 28 ppm (Chin, 2004). The clone has a sweetness Brix reading of 9.0%

Table 6: Yield and BSBD survivals of F1 hybrids in upscale field at ARC

Parents (CM)	Name of hybrid	Shoot yield (mt/ha/year)		Mean	Plant stand* as at Dec. (%)	Plant stand* as at Oct. (%)
Year		2004	2005		2005	2007
100 x 112/P2	'Eggie'	20.69	14.31	17.50	86	0.0
100 x 112/P1	'Waxie'	16.05	9.85	12.95	95	19.3
201 x 112/P4	'Cutie'	1.75	-	-	20	5.9
100 x 112/P2	'Greenie'	12.59	43.03	27.81	78	19.9
100 x 111/P1	'Wrinkie'	18.03	76.81	47.42	99	90.4
Mean	-	13.82	35.99	26.42	75.6	-

*Plant stand indicates live and productive plants.

Table 7: Traits* of five selected clones in the upscale field plot

Hybrid Name	Shoot weight (g)	Leaf weight x 10^{-2}(g)	Leaf/ Shoot Ratio	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Shoot Brix (%)	Leaf thickness x10^{-2} (mm)
'Waxie'	12.5	13	45.8	5.3	2.7	5.5	9.0	1
'Eggie'	15.8	19	42.4	5.3	2.9	5.0	10.0	2
'Wrinkie'	**12.5**	**12**	**47.9**	**4.5**	**2.2**	**5.5**	**9.0**	**2**
'Greenie'	17.1	16	47.8	5.9	3.1	5.0	8.0	1
'Cutie'	19.4	17	54.1	6.1	2.8	8.0	9.0	2

*Averaged of 10 shoot of 50 cm long harvested from experimental plot.

Eating quality

In breeding 'Wrinkie', the prime objectives are on the yield and BSBD resistance. Good eating quality of the final variety would be an added bonus. From the marketing point of view, this parameter is crucial for consumer acceptance. As this was not a varietal trial, no comparative taste evaluation was conducted. Quality was assessed by asking the 'desire to consume' to the staff at ARC after the first time eating it. For the farmers' trial sale markets at Serian and Sungei Maong, Kuching, we looked for returning buyers. Both approaches have yielded good acceptance and positive comments. There was no case of outright rejection. According to one farmer at Sungei Maong market, some customers even booked his 'Wrinkie' 'cekur manis' to ensure supply. 'Wrinkie' has been welcomed by 'Lui cha' and fast food stalls due to its acceptable eating quality, good sweetness and the ease of foliage crushing.

Foliage visual and physical attributes

The leaves are very dark green by visual comparison to other varieties, indicating the possibility of higher chlorophyll content. The intense green colour of cooked sweet shoot tends to persist longer than other varieties according to some consumers. This gives the impression that it is freshly prepared or cooked.

The crinkled leaves give a perception of bulkiness, even though measured leaf/stem ratios did not show a lot of difference. It gives you the sense of getting the 'money worth' of the vegetables. The wrinkled or crinkled appearance has a disadvantage as some new buyers might think that the shoots are diseased like those infested with insects and infected with viral disease. This could be overcome through explanation and getting acquainted with the vegetable.

Physical assessment testing by crushing the fresh leaves with fingers showed that this clone breaks easily under light twisting pressure and gives an audible breaking sound. This ease of breaking is an advantage as most people break the foliage by hand crushing before cooking. Furthermore, since the slightly older leaves are easily crushed, the edible portion per stick is increased. This makes 'Wrinkie' attractive to consumers, especially to the fast food vendors.

Table 8: Foliage and other characters of the 'Wrinkie' hybrid clone*

Growth habit	Uniform, upright, non-branching unless pruned.
Shoots	Slender, tapering with strong dark green foliage
Colour	Very dark green
Leaf physical attribute	Wrinkle, uneven, with shallow uneven depressions
Single leaf weight (g)	0.12 ±0.05
Leaf size (cm)	4.5 (l) x 2.2 (w)
Leaf thickness (mm)	0.02
Leaf stem juice (Brix)	9.0 %
Shoot physical attribute	Very crispy, easily crushed by hand when fresh
Time to harvest planted cutting	4 months onwards
Resistance to BSBD	Very resistant
Shoot yield (mt/ha/year)	12- 28

*Actual experimental records. Size may vary with environmental conditions and management.

FARMLET VERIFICATIONS

'Wrinkie' has been supplied to some farmers at Matang, Kota Sentosa, Taie, Siburan and Beratok for on-farm verifications. Overall, the farmers' responses have been positive, especially on disease resistance ability, yield and consumers acceptance at the vegetable markets. They have been supplying the markets at Serian, Sungei Maong and Kuching. At Taie, where the farmer planted several clones together, only 'Wrinkie' survived from the BSBD infection. Since then, he has been growing only 'Wrinkie' clone in the farm.

At Beratok, a Transfer of Technology (T.O.T) plot measuring 890 m^2 was established in early 2007. The pilot plot is now yielding 600 kg/harvest or the estimated annual yield of 80 mt/ha/year at 12 rounds/year (6.7 mt/ha/round). This is a very high yield for sweet shoots.

Records from the different locations indicated that 'Wrinkie' has yielded 36 to 80 mt/ha/year (Table 9). The frequency of harvest and cultural management would have affected the yield. The young shoots are normally harvested at 3 - 5 weeks intervals. To date no plant has died due to BSBD. The new F1 hybrid 'Wrinkie' has shown high tolerance or resistance to the disease under different field conditions and agronomic management in varying micro-climatic environments.

PLANTING MATERIAL SUPPLY AND SOURCE BUSH

The 'Wrinkie' clone is easily propagated by stem cuttings. Normally 25 - 30 cm long stem cuttings are sufficient to grow into healthy plants. At present, cuttings may be acquired free of charge at ARC, Tarat and Kebuloh stations. The farmers' plots at Siburan, Matang, Taie, Beratok and Kota Sentosa may also supply cuttings by arrangements. The genuine 'Wrinkie' source bush at ARC is able to supply some planting material for starting a source bush.

Table 9: Shoot yield of 'Wrinkie' in on-farm verification plots

Site	Plot size (m^2)	Harvest per round (g/m^2)	Projected shoot yield (mt/ha/yr)
Matang (Sinsantu)	60	600	72
Taie (Serian)	400	450	49
Kota Sentosa	500	300	36
Beratok	890	560	80

CONCLUSION

The F1 hybrid 'Wrinkie' clone, bred from local BSBD resistant and good eating quality accessions, has been proven as a productive clone with quality shoots and good BSBD resistance. As a new clone, 'Wrinkie' is expected to replace BSBD susceptible clones especially where the disease has been prevalent. In niche markets such as those destined for 'Lui cha' and 'fast food', 'Wrinkie' may have advantages due to high shoot yield, good sweetness, intense and persistent dark green colour after cooking and the ease of leaf crushing for cooking.

It may be concluded that the breeding programme for a superior and BSBD resistant sweet shoot has been successful. The new clone is strongly recommended for both new venture plantings as well as for rehabilitating the BSBD infected farms.

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