SWEET POTATO BREEDING METHOD USING WILD RELATIVES IN JAPAN

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The breeding of sweet potato, *I. batatas* (L.) Lam., is being promoted systematically in Japan: hybrid seeds produced at Ibusuki (Kyushu National Agricultural Experiment Station) are sent to Kumamoto (Kyushu National Agricultural Experiment Station) and Chiba (National Central Agricultural Experiment Station), and the promising strains selected there from seedling are distributed to the agricultural experimental stations of each prefecture where this crop is grown for future tests. The strains which show excellent performance at several places are finally registered as sweet potato varieties by the Ministry of Agriculture and Forestry and distributed to the farmers.

More than 2 million sweet potato seeds from known crosses have been produced at Ibusuki for 30 years since 1946, and 16 varieties such as "Koganesengan", "Benikomachi" or "Minamiyutaka' have been registered.

During the initial phase of sweet potato breeding, popular local varieties were mainly used as gene sources. In 1940 about 95% of the total area planted with sweet potato consisted of local varieties, but in the next 10 years over 80% was replaced by improved varieties bred through a systematic breeding program. This replacement by the improved varieties indicates that the breeding efficacy in the initial phase was considerably high. However, additional improvement of yield and quality becomes increasingly difficult to attain thereafter. The second marked genetic progress was achieved by the development of "Koganesengan" released in 1966, which includes exotic breeding materials. The introduced materials had played an important role in the genetic progress that had been made prior to this, especially by increasing the yield of tuberous roots and the starch content beyond the plateau achieved by domestic materials (Sakai, 1964).

A third noticeable change in yield levels was achieved by the development of "Minamiyutaka" registered in 1975. This variety, with one eighth of the germ plasm originating from the wild plant K123, surpassed the yield levels achieved by "Koganesengan" and other cultivars at several locations of the southern part of Japan.

Since 1956, many wild *Ipomoea* species have been introduced from the U. S. A., Mexico and other countries in Central America and the northern part of South America. Most plants introduced into Japan have been maintained at Ibusuki and some of them have been used to study sweet potato phylogeny as well as for the utilization of wild plants in sweet potato breeding (Nishiyama *et al.,* 1975, Sakamoto, 1970, 1976).

As seen in the development of "Minamiyutaka" and other materials including germ plasm of wild *Ipomoea,* the wild species have much to offer in the field of plant improvement. Although some contents of this paper have already been reported (Kobayashi and Miyazaki, 1976), the author wishes here again to demonstrate a practical method for sweet potato breeding using wild relatives.

Ipomoea species is closely related to the sweet potato.

Van Ooststroom (1953) recognized the taxonomic interrelationship between the sweet potato and its four closely related species grown in Malaysia by classifying them in the section *Batatas.* However, species identification was almost impossible because the species have not

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been well defined taxonomically. In addition, species of *Ipomoea* had been studied so little from the breeding viewpoint that one did not really know which species could be crossed with cultivated sweet potatoes.

More than 200 accessions of *Ipomoea* species have been introduced mainly from the area extending from Mexico to the Andes by several researchers. It was thought that the genetic resources of sweet potato included all materials that can be reached through hybridization and interest was directed toward wild plants that could be hybridized with the sweet potato, directly or indirectly.

Following hundreds of interspecific crossings, it was recognized that the *Ipomoea* species which can be hybridized with sweet potatoes have in common the following characters:

1) The flower is similar to that of sweet potato; the corolla is bell-shaped and not funnel-shaped, the colour of the interior of the tube is invariably darker than that of the limb, and the glands at the base of the corolla are prominent.

2) The plants are self-incompatible in general, and there are several incompatibility groups among them.

Based on the crossing ability with sweet potato or on the two above mentioned characters, assembled *Ipomoea* species of section *Batatas* have been divided into Group 1 and Group 2. Plants of Group 1 can be hybridized with sweet potatoes and plants of Group 2 can not. The accession numbers, species names and other characteristics of the wild plants belonging to Group 1 of section *Batatas* are shown in Table 1.

Table 1. Wild plants of Group 1, section *Batatas* maintained at Ibusuki

Accession number	Species*	Chromosome count	Geographic origin	Collector	(Year)
K221	*I. leucantha*	30	Mexico	M. Kobayashi	(1960)
K222	*I. (trifida 3x)*	45	Mexico	M. Kobayashi	(1960)
K233	*I. littoralis*	60	Mexico	M. Muramatsu	(1962)
K300	Unidentified	60	Ecuador	C. M. Rick	(1970)**
K400	Unidentified	60	Mexico	S. Shiotani	(1973)
K500	Unidentified		Colombia	M. Kobayashi	(1976)
K510	Unidentified		Colombia	S. Irikura	(1976)
K123	*I. trifida*	90	Mexico	I. Nishiyama	(1955)
K177	*I. trifida*	90	Mexico	M. Muramatsu	(1962)

* Identification of species was determined by Nishiyama and Teramura (Nishiyama 1961, Teramura *et al.* 1967)
** After F. W. Martin *et al.* (1974)

Breeding value of the wild species

It is generally assumed that wild species may have enormous value as sources of resistance or tolerance to many pests and diseases, and that one may be able to use the characteristics from wild relatives which enable them to tolerate physiological stress.

"Minamiyutaka" shows a strong resistance to root knot nematode and root lesion nematode, which come from a wild plant, K123, and tuberous roots of this variety have a larger storage tolerance than other cultivars. In addition to these desirable characters, the yield is higher than in any other existing varieties especially in the southern part of Kyushu (Table 2). The high yielding ability of "Minamiyutaka" was probably due to the genetic interaction of cultivated sweet potatoes and wild plants, K123.

Table 2. Yields of tuberous roots and starch of "Minamiyutaka" at several locations in the southern part of Kyushu (1974).

Locations	Yields per are of				Starch content
	Tuberous Roots		Starch		
	Weight	Ratio *	Weight	Ratio *	
	Kg	%	Kg	%	%
Takanabe, Miyazaki	337	113	82	106	24.3
Shintomi, Miyazaki	489	153	114	141	23.3
Sadohara, Miyazaki	442	147	107	149	24.1
Takahara, Miyazaki	416	120	93	111	22.3
Miyakonojo, Miyazaki	319	123	68	126	21.4
Akune, Kagoshima	442	112	101	113	22.8
Kagoshima, Kagoshima	466	134	101	132	21.6
Ei, Kagoshima	515	121	118	116	23.0
Osumi, Kagoshima	359	103	80	99	22.3
Mean, all locations	421	125	96	121	22.7

* The ratio of weight to that of "Koganesengan", a comparative variety, produced at each location.

Thus, the wild *Ipomoea* should provide useful genes for yield improvement of sweet potatoes. This is contrary to the situation observed in most crops. The breeding of sweet potatoes using wild relatives, however, has just begun and more must be learned about the genes or genetic complex which could be of value for sweet potato improvement.

Crossing technique

1 Flower induction

Flowering of wild *Ipomoea* maintained at Ibusuki is generally observed when days are short. Under the natural conditions of daylight prevalent at Ibusuki, most plants flower late in the season before the frost whether they are planted as seeds or vines from April through July, and some plants, which are one year or more old, do not flower or flower very sparsely. Therefore, an effective means of inducing flowering in *Ipomoea* plants would greatly facilitate the utilization of these plants for sweet potato breeding.

Flower induction techniques developed in our laboratory are satisfactory for this purpose. During any season, *Ipomoea* plants treated with this technique begin to flower within about 40 days. The outline of this method is as follows:

(1) Treat seeds of the dwarf type morning glory, *I. nil* (L.) Roth cv. Kidachi-asagao, with sulfic acid for 1 hour, keep in water overnight after rinsing, and then plant in 15 cm pots; (2) After germination, keep the pots under day-light conditions for about 1 month; (3) When seedlings are about 40 cm high and have about 10 leaves, cut off the stem tips and split the stem for insertion of the scion. The stem of the wild plants used as scions should be about 20 cm long and have cut 5-8 cm long on both sides of the stem; (4) Hold the grafts in place with grafting clips until the scion is established, and keep the grafted plants in a humid and sheltered place for about 1 week; and (5) Transplant the grafted plants to 24 cm pots and place them under favourable growing conditions. Table 3 shows an example of the effectiveness of this procedure.

Table 3. **Effect of grafting on flowering of wild *Ipomoea*, K300, planted on 7 July and grafted on 5 September (1974).**

Plant	Natural condition		Grafted on morning glory	
	Date first flowers opened	Number of flowers*	Date first flowers opened	Number of flowers*
K300-1	1 Nov	215	11 Oct	750
K300-2	3 Dec	32	11 Oct	432
K300-3	15 Oct	53	20 Oct	312
K300-4	–	0	26 Oct	303
K300-5	10 Dec	13	20 Oct	565
K300-6	–	0	13 Oct	331
K300-7	8 Nov	47	11 Oct	364

* Counting started when the first flowers opened and ended 31 Dec 1974, while the flowering continued.

2 Self-and cross-incompatibility

As far as we observed, the wild *Ipomoea* that would hybridize with sweet potato were all self-incompatible and several intra-incompatible, inter-compatible groups were recognized. It is, therefore, required to check the self-incompatibility and to determine the group before crossing. Self- and cross-incompatibilities of the wild *Ipomoea* were determined in our laboratory using the following staining technique.

Crosses were made under greenhouse conditions, keeping materials out of open pollination. The temperature of the greenhouse was kept at about 15°to 30°C. Flower pollinated before 10:00 while pollen from appropriate plants were collected 3 or more hours after pollination. Stigmas, with styles attached, were placed on glass slides and stained with a solution of 0.5% cotton blue in lactol phenol. A cover-glass was placed on the stigma and pressed with the fingers. The prepared slides were kept at room temperature for microscopic observation conducted the following day. Usually five flowers per cross were used for this purpose. The results from such tests are shown in Table 4, illustrating self- and cross-incompatibility relationships in wild *Ipomoea.* Compatibility of the wild relatives and sweet potato as observed in our laboratory to date are presented in Table 5. The schedule mentioned here, which is applied in research on pollination incompatibility of *Ipomoea,* makes it possible a rapid and correct interpretation.

Table 4. Pollen germination* on stigma in K300, indicating self-incompatibility and three incompatibility groups (1974).

Stigma	Pollen						
	K300-6	K300-7	K300-1	K300-2	K300-5	K300-3	K300-4
K300-6	–	–	+++	++	++	+	+
K300-7	–	–	+++	++	+++	±	+
K300-1	–	–	–	–	+++	±	+
K300-2	–	–	–	–	+++	+	++
K300-5	+++	+++	–	–	–	–	–
K300-3	+++	+++	+++	+++	–	–	–
K300-4	+++	+++	+++	+++	–	–	–

* Five stigmas per one crossing were observed. Over 50 pollen grains were put on each stigma. Symbols in the table: +++ more than 10 pollen grains germinated per stigma with four or five stigmas; ++ 6 ~ 9 pollen grains germinated; + 1 ~ 5 pollen grains; ± very rarely 1 ~ 3 pollen grains; – no pollen germinated.

Table 5. Relationship of incompatibility groups of sweet potato and wild *Ipomoea* (1958 ~ 1976)*

Accession	Plants tested	Determined groups in sweet potato**														Undetermined groups in sweet potato									Number of groups
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	1	2	3	4	5	6	7	8	9	
Sweet potato	>1000	O	O	O	O	O	O	O	O	O	O	O	O	O	O										14
K123	20	O	O	O	X	O	O	O	O							O	O	O							10
K233	7	X	X	O	X	X	O				O					X			O	O					5
K300	7	X	X	X						O	O					X									2***
K222	8	X	X	O	X	X															O	O			3
K221	5	X	X	O	X	X										X	–						O	O	3

* Incompatibility was determined by pollen germination on stigma.

** O = incompatible to sweet potatoes in each group; X = compatible.

*** Three incompatibility groups were classified in K300 (see Table 4), but two groups merged when tested with sweet potatoes.

3 Crossing ability and hybrid inspection

In most cases involving interspecific crosses of *Ipomoea,* it was frequently observed that no seed could be obtained even when the pollen germinated on the stigma. Therefore, it is necessary to check the real crossing ability by producing hybrid seeds. For this inspection, we cross at least 25 flowers for each mating combination. Apomixis and seed abortion are other problems to be solved. It is necessary to measure F_1 plants morphologically or sometimes cytologically to confirm hybrid identities.

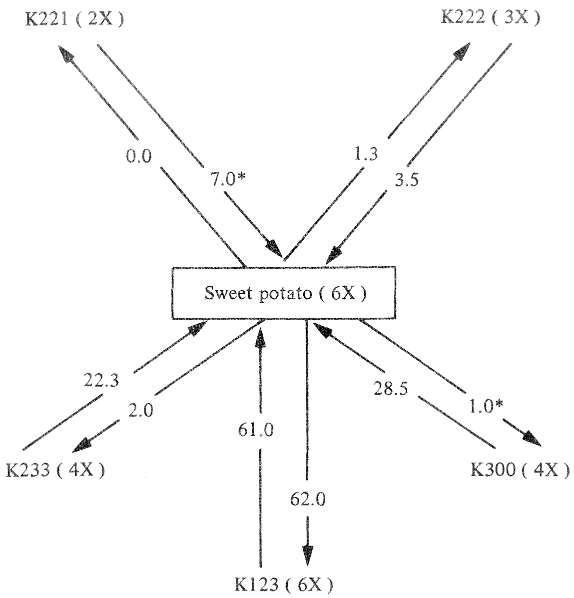

Fig. 1. Maximum seed set (%), from figures obtained between 1958 and 1976, in crossing of wild ***Ipomoea*** **and sweet potatoes.** Arrows point from male to female in crosses. Asterisks indicate that female was Kyushu 58, which includes one fourth germ plasm of K123.

Fig. 1 shows the maximum seed set percentages obtained so far at Ibusuki between wild *Ipomoea* and sweet potato. In some cases, we obtained only a few hybrid plants from more than ten thousand crossings. With the exception of K123, higher seed set (%) was obtained when the wild plants were used as male parents.

Gene introducing system

All plants of Group 1 section *Batatas* are considered to be important for the improvement of the genetic resources of sweet potato, although the breeding potential has not been completely evaluated. It was thought that the true meaningfulness of wild *Ipomoea* as genetic resource of sweet potato would be disclosed only after using it in sweet potato breeding. We tried several gene introducing systems, but we do not yet know which system is the most effective to obtain useful gene(s) from wild relatives.

In any system, back crossing would be effective in the later stage of the breeding program; the recurrent parents in back crossing should consist of different varieties of sweet potatoes. According to our experience, the following methods can be applied practically:

1. Direct use of wild plant. In our breeding program, K123, K222, K233 and K300 have been used directly. Wild plants with 90 chromosomes like K123 or K177 readily cross with sweet potatoes, and we can use them as donors in crossing procedures. From our experience, F_1's must be back crossed to sweet potato cultivars at least twice so as to breed the economical varieties. "Minamiyutaka" was bred by this method. We can also use K222 (2n = 45) directly as a donor like K123. Hybrids between K222 and sweet potato were found to be hexaploids. The seed set percentage in this case was extremely low, however, once a hybrid between triploid plant and sweet potato is made, back crossing thereafter will be rather easy since the chromosome number of the hybrid is identical to that of the sweet potato.

Because the sweet potato or hybrids propagate vegetatively, it is practical to use heteroploids with desirable characters. It is also possible to back cross heteroploids with more desirable characteristics. F_1's between K300 (2n = 60) and sweet potato have 75 somatic chromosomes and B_1's have been observed to have 82 or 84 chromosomes. We are now in the process of making B_4 generation. As the back cross numbers advanced, every character including the chromosome number became closer to those of sweet potatoes. Thus, we can use wild *Ipomoea* directly even if they are tetraploid or diploid.

2. Use of synthesized hexaploids. All plants of Group 1 section *Batatas* can be used directly as mentioned above, but in some cases low seed set and time created problems. To avoid these problems, we can use the artificial hexaploids synthesized from wild *Ipomoea* plants. Some theoretical ways of synthesizing hexaploids (2n = 90), some of which have actually been used for sweet potato improvement, consist of:

(1) Diploid $\xrightarrow{C}$ Tetraploid, Tetraploid x Diploid $\xrightarrow{C}$ Hexaploid
(2) Tetraploid $\xrightarrow{C}$ Octaploid x Tetraploid $\longrightarrow$ Hexaploid
(3) Diploid x Tetraploid $\xrightarrow{C}$ Hexaploid
(4) Triploid x Triploid $\dashrightarrow$ Hexaploid (in the case of outcrossing of K222)

$\xrightarrow{C}$: Doubled chromosome using colchicine solutions.
$\dashrightarrow$: Doubled chromosome by natural unreduced gametes.

It was found in our crossing experiments that seed set percentages from crosses between wild plants and sweet potatoes were higher when the synthesized hexaploids were used as female parents. Besides these systems of synthesized hexaploids, triple or quadruple hybrids having 90 chromosomes are used as gene sources for sweet potato breeding. Wild *Ipomoea* does not possess many desirable characteristics in one species. However, it is possible to combine useful characteristics in different species for triple or quadruple hybrids.

3. Use of lower ploidy. By taking advantage of the lower ploidy of wild *Ipomoea* we have obtained tetraploids with enlarged storage roots. These "Tetraploid Sweet Potatoes" have been bred using K221, K222 and cultivated sweet potatoes. A very wide variability was recognized among the tetraploids for all characters observed, and some tetraploids produced tuberous roots which were close to those of usual sweet potato varieties. We are trying to breed a variety which ripens early in using tetraploids.

Summary and conclusions

An attempt to introduce some useful germ plasm of wild *Ipomoea* into sweet potato has been systematically promoted in Japan since 1956. For this purpose, more than 200 accessions of *Ipomoea* species have been introduced and used for crossing experiments at Ibusuki. Plants in the section *Batatas* genus *Ipomoea* were divided into two groups based on their crossing ability with cultivated sweet potatoes. Plants of Group 1, which can be hybridized with sweet potatoes, include diploids, triploids, tetraploids and hexaploids. All of them resemble the sweet potato in two respects: they have similar floral morphologies and incompatibility systems.

To utilize wild species in a sweet potato breeding program, a flower induction technique and self- and cross-compatibility tests were worked out. Some principles necessary for an effective gene introduction system have been identified.

The breeding potential of wild species has not been fully evaluated, however, a variety and various breeding materials including germ plasm of wild *Ipomoea* indicate that wild species may provide genetic resources for yield improvement in addition to disease resistance and insect tolerance.

A critical understanding of wild *Ipomoea* characteristics could lead to greater advances in sweet potato improvement.

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Discussion

N. Murata, Japan: I would like to know more about exchanges of cultivars among various countries for breeding purpose. Is it possible to use your cultivars in other countries as gene sources? Could promising cultivars be introduced from abroad? Are there many important characteristics present in wild species but not in exotic cultivars?

Answer: It is important to introduce exotic cultivars for yield improvement. Heterosis can be expected to be found in wild species. Wide scale screening would be valuable in the case of blackrot which is the most important disease in sweet potato, as gene source of resistance to this disease has not yet been found.

J. T. Rao, India: What are the undesirable characters that you are likely to get from the wild types and how do you propose to eliminate them?

Answer: Generally speaking, two sequences of backcrossing are needed for this purpose. In the case of "Minami Yutaka", three sequences of backcrossing were carried out to eliminate some of the undesirable characters present in the wild plant K123.

Y. Watanabe, Japan:

1. To what extent have the genomic relationships been clarified in the genus *Ipomoea?*

2. Are you aware of any cytoplasmic effect of wild species "Trifida" on the agronomic characters?

3. You are going to breed 4x sweet potatoes. What is the advantage of reducing the ploidy?

Answer:

1. There are three kinds of plants in the Section *Batatas,* genus *Ipomoea.*: G1, 2 and an intermediate between 1 and 2. The genome of G1 is B, of G2, A and that of the intermediate type has not yet been identified.

2. I have not studied this aspect.

3. By reducing the ploidy from 6x to 4x, one can expect early maturity, better taste and quality.

C. Kaneda, Japan: What would happen if you used a single variety as recurrent parent? Do you use different varieties from the standpoint of just getting greater variation among offspring plants?

Answer: If we used a single variety as recurrent parent, yield level of offspring plants would fall down rather rapidly when the number of backcrosses increases owing to inbreeding depression. The idea of using different varieties as the recurrent parent is aimed at using heterosis effectively in the breeding procedures.