Flower Induction by Top-Grafting in Sweet Potato

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Abstract

After three decades of practical experience and formal tests, the "top-grafting method" can now be recognized as the most effective and handiest technique of inducing the sweet potato to flower and undergo seed-set especially under greenhouse conditions when time is limited to six months or less.

As a stock plant, a dwarf type of Japanese morning glory, *Ipomoea nil* (L.) Roth cv. Kidachi-asagao, was found to be most effective in inducing flowering. The top-grafting method used was presented.

It is believed that the top-grafting method as outlined, would greatly facilitate sweet potato breeding even in the tropics when poor flowering varieties are used.

Introduction

Flower formation of sweet potato, *Ipomea batatas* (L.) Lam., is in general, related to latitude; it flowers frequently in the tropics while little or no flowering occurs in temperate regions at latitudes higher than 35°N or S. Even in the tropics, the intensity of flowering varies widely with seasons from poor to abundant, and some varieties produce little or no flowers at all (Schreven 1954; Rheenen 1965). It is, therefore, necessary to induce flowering artificially for breeding purposes especially in temperate regions of the world.

It is generally recognized that a short photoperiod, a slight humidity, use of certain chemicals, use of certain *Ipomoea* rootstocks, or proper nutrition, promote sweet potato to flower. Many flower-inducing techniques based on these phenomena have been developed and employed for sweet potato breeding.

In Japan, our laboratory at Ibusuki (31° NL) has been in charge of producing hybrid seeds of sweet potatoes since 1944 and more than two million seeds from known crosses have been made for breeding use.

Studies on inducing flowering for breeding purposes in sweet potato and its relatives have been done in our laboratory during these three decades. We are now using a "top-grafting method" which seems the most effective and handiest.

This paper represents a summary of our knowledge and findings on flower stimulation in sweet potatoes and presents a top-grafting method which greatly facilitates sweet potato improvement.

Outline of Hybridization in Sweet Potatoes

One of the early reports of sexual reproduction of sweet potato, according to Thompson (is cited by Rao 1961), is a report of the work done at the St. Helena Penal Colony in Queensland in 1901. Since then, similar attempts at breeding this crop have been done in several places.

The breeding program which started in 1914 and was carried through 1944 at the Okinawa Agricultural Experiment Station was probably the most extensive and also most successful in developing new varieties through hybridization (Iura 1951). Originally, a complete variety breeding program was conducted at the Okinawa station, but after 1919, work was limited to the production of breeding materials (hybrid seeds and the first year seedling selections) by the Japanese Government. After that time, hybrid seeds produced in Okinawa every year were distributed to several places in the mainland of Japan for further selection. In 1944, the Ibusuki Branch of Kagoshima Agricultural Experiment Station assumed the responsibility for producing sweet potato seeds previously done in Okinawa. In 1947 this project was transferred to the National Agricultural Experiment Station where it continues to date.

When the hybrid seed production work was transferred to Ibusuki more than 65% of the sweet potato clones did not flower even under greenhouse conditions though considerable research on inducing flowering in sweet potatoes has been done by earlier researchers (Wada, 1923; Hirama, 1929; Noda, 1936; Kojo, 1937; Shigemura et al., 1938; Sugawara, 1938, Akimito, 1939; Fujise et al., 1955).

Similar work on sweet potato flowering was carried out in the U.S.A. by Miller and other researchers (Miller, 1937, 1939; Mikell et al., 1948; Kehr et al., 1953; Lam et al., 1955). Programs to develop new sweet potato varieties through hybridization were started in Louisiana and other states.

As for flower inducing techniques for breeding purposes, several methods such as short days, grafting, water culture, chemical application, root pruning, vine girdling, growing the vines on trellises, or other methods were proposed by many workers in 1940.

After comparative studies on flower induction by the techniques mentioned above, a grafting method with short day treatments was adopted and gradually improved for seed production in Ibusuki (Fujise et al., 1955; Ando et al., 1963; Kobayashi et al., 1976, 1978). The main techniques for flower production used in out laboratory during the last three decades are given in Table 1.

A Change of Flower Induction Techniques

At first, evening glory, *Ipomoea alba* L., was employed as the rootstock for inducing sweet potato flowers. Although *Ipomoea alba* L. appeared to have a lower ability to induce blooming than that of the morning glory, *Ipomoea nil* (L.) Roth, was used as a rootstock for two reasons: i) grafting manipulation is easier than with the morning glory since the stems of *I. alba* are much thicker, and ii) the flowering period is longer so that the total number of flowers is increased.

The trouble with using *I. alba* rootstocks was that the flowering time varied with parental clones, and some sweet potato varieties did not produce any flowers after grafting. To overcome these difficulties, a "Double Method" was devised. In this method sweet potatoes were grafted onto *I. alba* first, then placed under artificial short day conditions with a photoperiod of 10 hours. The double method combined the grafting and short day treatments and contributed considerably to hybrid seed production in our

laboratory in difficult-to-flower varieties.

About 1955 a dwarf type of Japanese morning glory, *Ipomoea nil* (L.) Roth Cv. Kidachi-asagao (M. Kobayashi and T. Miyazaki, 1976) was found to be a promising rootstock for sweet potato flower induction by Imamura at the Kyoto University. This unique morning glory was introduced into our laboratory in 1957 (Fujise, 1964).

Grafting tests showed that 'Kidachi-asagao' was more effective in inducing flowering than *I. alba,* and also had a thick stem which made grafting easier. 'Kidachi-asagao' thus took the place of *I. alba* as the rootstock for inducing sweet potatoes to flower in our laboratory after 1958. Plants of 'Kidachi-asagao' with cotyledons only were used as rootstocks in those days. The double method using 'Kidachi-asagao' rootstock and short days was employed for about five years.

The accumulation of carbohydrates in the grafted scions was believed to induce flowering by early researchers (Miller, 1937; Akimoto et al., 1939; Kehr et al., 1953), and plants of *I. alba* or *I. nil* without foliage leaves were used as the rootstocks in our laboratory.

However, as knowledge of the physiology of flowering in plants advanced, the effects of leaves remaining on stock plants were taken into consideration (Lam et al. 1955). Thus, several tests of "Top-grafting" in which scions were grafted on the top of the morning glory 'Kidachi-asagao' with several active leaves were also done in our laboratory (Ando et al., 1963; Fujise, 1964; Kobayashi et al., 1976). As a result, top-grafting was found to be more effective than the double method using 'Kidachi-asagao' rootstock without leaves and short days.

After 1963, top-grafting, using 'Kidachi-asagao' stocks, was used for breeding purposes and continues to be employed in our laboratory.

Table 2 indicates the approximate days required for flowering by the three methods.

'Kidachi-asagao' and Its Use for Grafting

'Kidachi-asagao' which means literally "a morning glory growing like a tree," is an extremely dwarf type of morning glory. Its origin is uncertain, but is presumed that this unique variety was bred at the beginning of the 1800's in Japan when many mutative varieties of morning glory were developed or found by fanciers.

We have now several strains called 'Kidachi-asagao', which vary a little in flower color, plant height, leaf color or shape, etc. From grafting tests using five different strains called 'Kidachi-asagao' in 1976 to 1978, the strain which has been used in our laboratory since 1957 was found to be the most effective in inducing sweet potato flowering. Therefore, 'Kidachi-asagao' refers to that strain in this paper.

Botanical characters of 'Kidachi-asagao' are the same as other popular morning glories seen in Japan except for the following three characteristics: i) plant height, ii) thick stems, and iii) flowering habit. Plant height – is usually about 15 cm under natural conditions while other morning glories grow about 3 m or more. 'Kidachi-asagao' was regarded as a mutant involving biosynthetic processes of gibberellins (Hirono et al. 1960). Stems of normal morning glories show a twining habit, but 'Kidachi-asagao' has a straight vine tip. 'Kidachi-asagao' produces a thick stem averaging 5 mm in diameter in contrast with 2 mm for normal morning glories. Normal morning glories are considered to be short-day plants, however, 'Kidachi-asagao' is day neutral; it produces many flower buds even under continuous light conditions. Three to five flower buds come out at all nodes from the first node to the final one if cultivated at temperatures of 25° to 35°C. Table 3.

shows plant growth of 'Kidachi-asagao' under natural- and long-day conditions with and without flower buds.

Sweet potatoes can be made to flower at any season with top grafting using 'Kidachi-asagao' stocks. However, the stock and scion plants are prepared twice a year, spring and fall, mainly because it takes about six months to complete one cycle of sweet potato seed production. It takes about two months for stock and scion plant growth, two months for crossing and one month for seed maturation. We make a total of about 3,000 stock plants a year using a greenhouse with an area of about 650 m^2.

Cultivation of 'Kidachi-asagao' for grafting use has been done in our laboratory.

The seeds of 'Kidachi-asagao' are treated with sulfuric acid for 30 minutes. When seeds stored more than two years are used, scarification requires one hour or more.

After scarification, treated seeds are rinsed with water overnight, then planted two seeds per 15 cm pot containing fertile soil. The pots are placed under the following greenhouse conditions: temperature, 20-35°C; humidity, 60-70%; daylight intensity, 50% cut using cheesecloth; and daylength, 16 hours or more. One plant is removed after germination leaving one vigorous plant in each pot. Nitrogenous fertilizer, usually a spoonful of oilcake mixed with ammonium sulphate, is applied per pot after about one week.

Foliage leaves come out about two weeks after planting, then flower buds are formed at each node as the plants grow. Flower buds should be removed since their development restrains plant length. Longday treatments reduce flower bud formation to some degree, but flower buds are taken off at least two times during the growing period of 'Kidachi-asagao'. When seedling heights of 'Kidachi-asagao' reach about 40 cm with 15 or more leaves, they are used as the grafting stocks. About two months are required for stock growth (Table 3).

Grafting is done as follows: stem tips of 'Kidachi-asagao' are cut off and the stem split with a razor blade for insertion of the scion. The stems (10-15 cm long) of sweet potato used as scions, properly cut on both sides, are inserted and held with grafting clips or twine. Grafted plants are kept in a humid and sheltered place (humidity, 90%) for 7 to 10 days until the graft union is established. Rather than using humidifiers in the greenhouse, polyethylene bags around stock and scion are used until establishment of the union.

Through this grafting procedure, successful unions are obtained in almost all plants treated in our laboratory. We can make about 30 grafts per hour.

The grafted plants are transplanted to 24-cm pots and the sweet potato scions are trained to four props put in each pot as they grow. The pots are usually placed on racks in the greenhouse for crossing experiments.

In most sweet potato varieties treated by this method, flower buds appear in about three weeks. They continue to flower for more than two months making a total of some 150 flowers per plant, The total number of flowers induced by top-grafting varies with varieties and individual plants.

Although top-grafting using 'Kidachi-asagao' stock plants seems to be the best treatment in regard to flower numbers produced in a limited time, we still have a problem with anti-affinity when some varieties are used as scions.

The following techniques have been found effective for eliminating that problem:

i) Burying of grafted parts. Time of occurrence of anti-affinity for grafting is indefinite; some sweet potato scions begin to wither 4 weeks after grafting, and others two months after grafting resulting in death of scions and stocks. Withering scions are revived by burying the graft union in soil if the scion plants are still alive. Most flower

buds which are formed on scions before wilting will open. Although new flower buds are not produced by this method, the anti-affinity problem is evaded in some degree.

ii) Shoot tip grafting. Until recently, we used sweet potato terminals of 10 to 15 cm as grafted scions for the usual top-grafting. However, it was found that shorter scions produce more flowers than longer ones. Also, grafting anti-affinity scions was restrained by the use of shorter scions (Nakanishi et al. 1979).

Tables 4 and 5 show that about twice the number of flowers were produced on scions originating from shoot tips (1 cm long) as on the usual ones (14 cm long). Although the grafting operation is somewhat difficult, shoot tip grafting is especially useful when poor flowering varieties are used as well as for reducing anti-affinity of grafting.

iii) Sweet potatoes improved for stock use. Since 1977 we have been working to improve sweet potato to be used as a stock plant instead of the morning glory, 'Kidachi-asagao'. Our main breeding objectives for this sweet potato variety are: high flower inducing ability, complete grafting affinity, dwarf type with many leaves, variegated leaftype, and white flower bearing. We have already obtained some promising lines showing progress toward that breeding goal.

Acknowledgment

The authors wish to express their sincere thanks to Dr. Alfred Jones, Research Geneticist of SEA, USDA, for his kindness in reviewing and correcting the manuscript.

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Table 1. Transition of flower induction methods applied to sweet potato seed production at Ibusuki

Year							Approx. No. (x 1,000) of seeds produced	
1946	1950	1955	1960	1965	1970	1975	Total	Yearly mean
Grafting using I. alba rootstock							215	31
		Double grafting using I. alba rootstock					124	21
				Double grafting using 'Kidachi-asagao' (I. nil) rootstock			195	49
				Top-grafting using 'Kidachi-asagao'			1,562	98

——— Mainly use
------ Partly used
– – – Rarely used

Table 2. Approximate days required for preparation of stock plants and flowering achieved by the different methods

Method	Stock plant Growth	Days of first flowering after grafting
Top-grafting using 'Kidachi-asagao'	45 – 60	30 – 45
Double method using I. alba	20 – 25	100 – 120
Grafting using I. alba	20 – 25	120 – 160

Table 3. Effects of day length and flower bud presence on plant length and number of foliage leaves of 'Kidachi-asagao' planted 12 January, 1979

Day length	Flower buds	Plant length (cm)		Number of foliage leaves	
		15 Feb	8 March	15 Feb	8 March
Natural (ca. 10hr)	Present	5.8	7.1	5.4	7.4
	Removed	5.5	8.4	5.4	11.0
Long day (ca. 16hr)	Present	5.0	29.5	6.4	13.2
	Removed	5.8	41.2	5.8	13.8

Variety 34 'Kidachi-asagao' Grafting* Day length** After grafting flowers

Table 4. Flowering effects of stock, grafting method, and day length on poor flowering sweet potato varieties

Variety	Stock used	Grafting* method	Day length**	Days of first flowering after grafting	Total number of flowers
Kanto 34	Evening glory	base	natural	54	23
do.	(I. alba)	do.	short	159	2
do.	'Kidachi-asagao'	top	natural	28	88
do.	(I. nil)	tip	do.	29	170
do.					
Gifu 1	Evening glory	base	do.	146	5
do.		do.	short	— —	0
do.	'Kidachi-asagao'	top	natural	28	100
do.		tip	do.	27	141

* base, stocks without foliage leaves used; top, stocks with foliage leaves used; tip, shoot tips of about 1cm long were grafted onto stocks with foliage leaves.

** natural, day length was between 11 to1 14 hours; short, 8 hours.

Table 5. Effect of scion length on number of flowers three months after grafting*

Variety	Scion length (cm)		
	≥14	2	1
Gokokuimo	86	119	134
Shirosengan	121	149	165
Norin 6	87	195	290
Norin 8	128	249	263
Norin 9	101	128	200
Kanto 33	68	150	193
Kanto 38	154	107	140
Kanto 40	75	153	116
Kyukei 19-1001	187	219	237
Average	112	163	193

* Scions were grafted onto 'Kidachi-asagao' stocks.