

## REVIEW

# Recent Progress in Breeding Low-temperature-tolerant Sweetpotato Lines in Japan

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### Abstract

Sweetpotato is among the most important crops originating from the tropics. Low soil temperature inhibits the thickening of sweetpotato roots. The growth of ordinary sweetpotato cultivars is thought to be limited below 15-16°C. In Japan, commercial sweetpotato production has recently been attempted in cool areas, in addition to warm areas, and a device for testing low-temperature tolerance in sweetpotato has been developed. In this system, containers fabricated from aluminum boards were filled with horticultural soil and set in a cooled water tank. Sweetpotatoes were cultivated at a soil temperature of 17°C for three weeks. Some lines exhibiting slight growth in low soil temperature conditions (14°C) were successfully bred, and achieved high yield from early planting in fields. It is important to breed low-temperature-tolerant sweetpotato lines achieving both high yield and high quality, in order to expand the crop's cultivation range.

**Discipline:** Crop Science

**Additional key words:** cool area, selection, soil temperature, testing device

### Introduction

Sweetpotato (*Ipomoea batatas* [L.] Lam.) is one of the most important crops originating from the tropical area of Central and South America, specifically from Mexico to Peru (Komaki 2001). About 8,500,000 hectares are planted globally, producing around 110 million tons of tuberous roots per year. In Japan, sweetpotato cultivation spread gradually after being introduced in the 1600s, increasing to a maximum of 440,000 hectares nationally. With about 36,000 hectares of currently planted area in Japan, a little less than 900,000 tons of sweetpotato are produced per year (Ministry of Agriculture, Forestry and Fisheries 2016). Although the total area of cultivation has subsequently declined, sweetpotato is among the staple crops grown in the upland farming zones of southern Kyushu and the Kanto region. Kagoshima prefecture accounts for one-third of

Japanese sweetpotato production, with Ibaraki and Chiba prefectures also being major producers. In recent years, the commercial production of sweetpotato has been attempted in the cooler regions of Hokkaido and Tohoku. Public institutes have also initiated agronomical research on sweetpotato in these areas.

Given its origination from a tropical area, sweetpotato encounters such common obstacles as etiolation of the leaves and death of the growing point (epicotyl) when subjected to low temperatures during growth. When sweetpotato damage is not ignored, the limited growth and yield of the crop can be observed. In order to expand its cultivation range, sweetpotato plants must be able to tolerate low temperatures. One potentially effective method of achieving this is breeding low-temperature-tolerant sweetpotato cultivars. This paper describes the current situation regarding the breeding of low-temperature-tolerant sweetpotato lines in Japan.

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## The effect of low temperature on sweetpotato growth

There are several reports about the effect of low temperature on the growth and formation of the tuberous roots of sweetpotato. Ueki et al. (1987) reported that the formation of tuberous roots was best under an intermediate (25°C) soil temperature condition, and that the thickening of roots was inhibited under low (15°C) soil temperature. Eguchi et al. (2003) reported that the growth of fibrous roots was markedly inhibited under a low (16°C) temperature condition. However, they did not observe such problems as the etiolation or discoloration of leaves at this temperature, but concluded that the temperature was low enough to limit sweetpotato growth. As a result, temperature of 15°C or 16°C is considered the lower temperature limit for ordinary sweet potato cultivar growth.

There is one report that discusses the role of genetic functions in limiting sweetpotato growth. Noh et al. (2009) cultivated sweetpotato plants for four weeks under a 12°C or 28°C temperature condition, and observed that low temperature significantly decreased the growth of the stem, petiole, leaf, and roots. They concluded that this decrease was caused by a reduction in the elongation growth of the epidermal cells, and not by a decline in cell number. They observed an increase in root thickness for plants grown at 12°C, suggesting that cell division was elevated. Genetic analysis revealed that the expression of three sweetpotato expansin genes (*IbEXP1*, *IbEXP2*, *IbEXPL1*) were ultimately down-regulated under the 12°C temperature condition. Hopefully, genetic analysis will achieve more advances in the future.

Several reports explore the low-temperature tolerance of sweetpotato from a physiological perspective. Lin & Block (2009) investigated methods of reducing chilling injury in sweetpotato plants. Pretreatments with both H<sub>2</sub>O<sub>2</sub> and NaCl solutions, adjusted by moderate density, reduced chilling injury in sweetpotato shoots after three days under a 2.5°C temperature condition. They concluded that moderate stress imposed as a pretreatment increased plant tolerance to subsequent chilling under specific conditions. Sato & Saruyama (2006) reported that pretreatment of heat shock in rice plants also increases low-temperature tolerance. Thus, there may be a similar method of increasing the low-temperature tolerance of sweetpotato. However, the costs of such methods must be reduced for facilitating application to sweetpotato cultivation areas. There is a series of papers discussing the low-temperature tolerance of tuberous roots, such as Uritani & Yamaki (1969), which reported that the degree of unsaturation of fatty

acids in mitochondria was lower in sweetpotato than in white potato.

## The response of sweetpotato cultivars or lines to low temperature stress

The response of sweetpotato cultivars or lines to low temperature stress has been reported previously. The low-temperature tolerance of sweetpotato tuberous roots was investigated in the cultivars “Okinawa-100” and “Norin-1” (Uritani & Yamaki 1969). They found that “Okinawa-100” had low tolerance and “Norin-1” had moderately high tolerance. Furthermore, Nakatani et al. (1986) found that rooting of the cut-sprouts of the cultivar “Okinawa-100” occurred normally at temperatures ranging from 19°C to 37°C and gave the maximum value at about 30°C. Nakatani et al. (1989) investigated varietal differences in optimum soil temperature for rooting under controlled soil temperature conditions, using 12 cultivars or lines. They found that the ratio of total root length under 18°C to that under 28°C was higher in the cultivars “Norin-1” and “Minamiyutaka,” and lower in the cultivars “Tamayutaka,” “Norin-2,” “Shiroyutaka,” and “Shirosatsuma.” They also reported differences in total root length under a critical soil temperature (15°C) (e.g., higher in “Norin-1” and “Minamiyutaka”). They concluded that these cultivars had higher rooting ability under low soil temperature.

## Improvement of low-temperature tolerance of sweetpotato through somaclonal variation or introduction of genes

Ueno et al. (2000) reported improved low-temperature tolerance of sweetpotato through somaclonal variation, following cell line selection. They used “Kokei-14” (i.e., a major cultivar for table use) and selected cell lines with higher tolerance to chilling stress, as compared with non-selected sweetpotato callus when cultured at -1°C for seven days. The regenerated plants were exposed to a low temperature (4°C for seven days), and some of the selected clones tolerant to chilling exhibited normal growth with no necrosis.

Breeding low-temperature stress-tolerant sweetpotato lines by introducing resistant genes is also discussed in related literature. Kasukabe et al. (2006) successfully developed low-temperature tolerant lines from the original “Kokei-14” cultivar by introducing the *FSRDI* gene (i.e., spermidine synthase gene), thereby increasing the low-temperature tolerance. Lim et al. (2007) also developed transgenic sweetpotato plants (SSA plants) with an enhanced tolerance to multiple

stressors, by expressing the genes of both CuZn superoxide dismutase (*CuZnSOD*) and ascorbate peroxidase (*APX*). The SSA plants treated with a chilling stress (entailing exposure to 4°C for 24 hours) exhibited little decrease in photosynthetic activity relative to the original plants. After a 12-hour recovery period, the photosynthetic activity of the SSA plants recovered fully, whereas the original plants' activity remained at a low level. Kim et al. (2009) developed transgenic sweetpotato plants expressing the *Arabidopsis* nucleoside diphosphate kinase 2 (*AtNDPK2*) gene, and the plants showed enhanced tolerance to cold stress. They reported that overexpression of *AtNDPK2* in sweetpotato might efficiently modulate oxidative stress from various environmental stresses. As mentioned above, utilizing somaclonal variation or genetic modification could lead to plants with tolerance to chilling stress. However, producing new sweetpotato cultivars with chilling stress tolerance at the market level remains difficult. One concern is that consumers avoid genetically-modified crops. Consequently, Kuranouchi et al. have proceeded with breeding sweetpotato plants with a high tolerance to chilling stress by crossing genetic lines and through subsequent selection utilizing a new testing device.

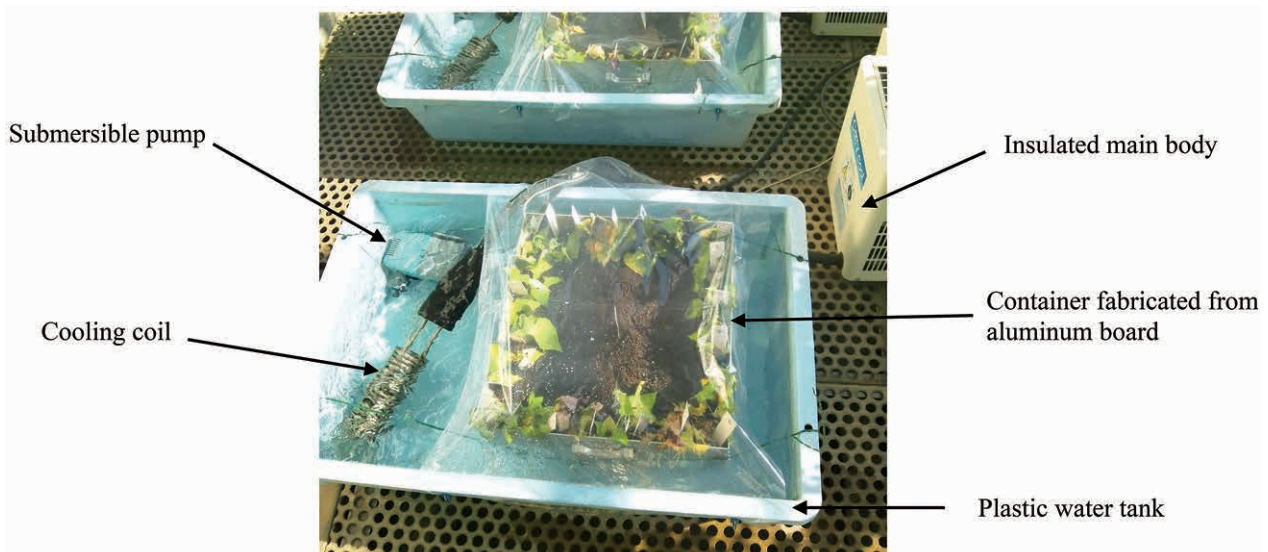
### Development of a device for testing the low-temperature tolerance of sweetpotato

There are some reports about devices that can control soil temperature during the cultivation of sweetpotato plants. Ueki et al. (1987) controlled soil temperature by setting stainless steel pots in a water tank

with controllable temperature. Eguchi et al. (1994) experimentally produced a system that can precisely control the temperature of soil both above and below ground, in order to determine the most suitable soil temperature for sweetpotato growth and the formation of tuberous roots. In this system, sweetpotato plants were grown by sand culture.

Kuranouchi et al. (2008a) produced a water culture method to evaluate the low-temperature tolerance of sweetpotato plants using growth chambers capable of eight levels of simultaneous and precise temperature control. In this method, the tips of vines (with 3-4 leaves) were grown using a water culture under three temperature conditions (10°C, 15°C, and 25°C) in growth chambers for three weeks. They measured the weight, number of leaves, ratio of dead leaves, and root number of the vines. Among the three temperature conditions tested, 10°C was found to be the best to differentiate sweetpotato lines for low-temperature tolerance in water culture. The difference in growth of the sweetpotato lines was not clear when grown under the 15°C and 25°C conditions. However, when the plants were grown at 10°C, insufficient rooting made it difficult to check the rooting ability.

Kuranouchi et al. (2008b, 2019) also developed a low-temperature testing device that more closely reflects real cultivation in fields than water culture. Figure 1 shows the appearance of this testing device. It has a simple structure compared to the devices reported by Ueki et al. (1987) and Eguchi et al. (1994). Moreover, it is suitable for breeding low-temperature tolerant sweetpotato plants, because it can treat many lines at one



**Fig. 1. Low soil temperature testing device**  
Kuranouchi et al. (2019)

time. A plastic water tank (measuring 85 (W) x 55 (D) x 20 (H) cm) filled with water was set in a greenhouse, equipped with a cooling coil (Orion Co., Carry Cool LPA2-J) to control the water temperature. A small water pump circulated water around an aluminum board container (measuring 40 (W) x 43 (D) x 15 (H) cm) that was placed within the plastic tank. This container was filled with horticultural soil moistened with water. Sweetpotato vines were planted at a depth of about 5 cm, so that two or three nodes of the vines were buried approximately 1 cm inside the wall of the aluminum container as shown in Figure 2. Daily mean soil temperature ranged from +1°C to -0.5°C, compared to the preset temperature. Daily mean soil surface temperature ranged from 4°C to 7°C higher than the mean soil temperature, and was greatly affected by the weather.

Sweetpotato vines of most cultivars and lines died when grown at 10°C in this device. Then the growth of vines under three temperature conditions (14°C, 17°C,

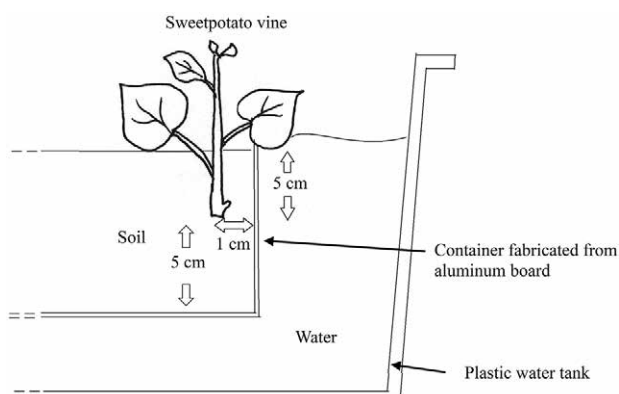


Fig. 2. Illustration of the method of planting Kuranouchi et al. (2019)

and 20°C) were observed using the same device. As a result, Kuranouchi et al. (2008b, 2019) concluded that a soil temperature of 17°C was most suitable for the selection and three-week cultivation period (Fig. 3).

### Selection of low-temperature-tolerant sweetpotato lines

From 2002 to 2006, Kuranouchi et al. (2007) cultivated different sweetpotato cultivars and lines to study their adaptability to slightly low soil temperature conditions, at an altitude of around 350 m. The sweetpotato line “S1-14” demonstrated good initial growth and high yield, and thus was thought to be promising. This line was bred by Mr. Umemura (a former researcher of the Hokkaido National Agricultural Experiment Station) and derived from a cross using a parent line originating from South America (Kuranouchi et al. 2019).

Vines of “S1-14” showed some growth even under a low-temperature condition (14°C) and grew vigorously at 17°C when cultivated in the low-temperature testing device (Kuranouchi et al. 2019). Conversely, the vine growth of many cultivars and lines was inhibited at 14°C. Some vines belonging to these cultivars died under this temperature condition. When cultivated at 17°C, the vines grew moderately or slightly. “S1-14” was thought to be promising as a low-temperature tolerant line, although this line’s quality characteristics were insufficient. As a result, Kuranouchi et al. used “S1-14” as a parent line for breeding. The line “00LT01LS3” developed from “S1-14” showed the same or slightly higher tolerance to low-temperature conditions as the parent lines (Kuranouchi et al. 2008a).

Kuranouchi et al. subsequently continued screening

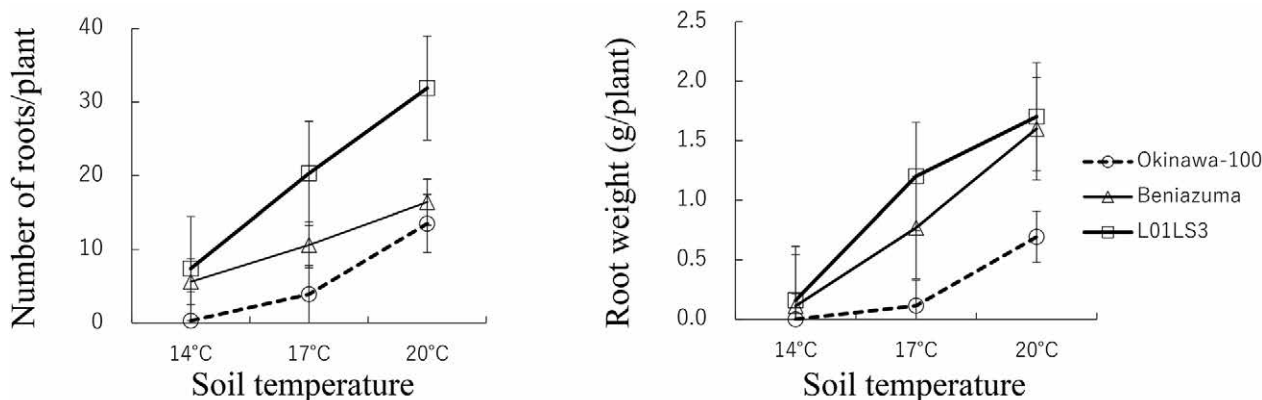


Fig. 3. Number of roots and root weight of the three sweetpotato varieties and lines after 3-week cultivation under low soil temperature conditions in 2010. Vertical bars indicate the standard error. Kuranouchi et al. (2019)

**Table 1. Initial growth and root yield of low-temperature-tolerant selected lines (2009)**

Cultivar/line	Initial growth		Yield	
	Vein weight (g)	Root weight (g)	Vein weight (g)	Root weight (g)
Beniazuma	39	7	622	378
Okinawa-100	18	4	693	375
S1-14	34	7	382	385
00LT01LS3	41	5	874	518
Selected lines				
03239-311	47	15	790	776
06188-26	80	22	750	739

Notes: Initial growth: About two months after planting

Yield: About four months after planting

Beniazuma: Standard cultivar in Japan, with medium low-temperature tolerance

Okinawa-100: Low-temperature tolerance is low.

S1-14 & 00LT01LS3: Low-temperature tolerance is high.

Data are shown by weight per plant.

Modified from Kuranouchi et al. (2010)

from worldwide genetic resources of sweetpotato using this low-temperature testing device, and have found some tolerant lines. These lines have been used as parents to breed new tolerant cultivars and lines. Among them, the line “06188-26” showed high tolerance to low-temperature conditions with a comparatively high root yield during early planting (Table 1, Kuranouchi et al. 2010). However, the cooking quality of “06188-26” is insufficient and this characteristic must be improved. Newly bred low-temperature-tolerant lines showed high yield during trials in Hokkaido prefecture, the northernmost part of Japan, demonstrating the effectiveness of the above selection method (Kuranouchi et al. 2014).

## Conclusion and future breeding aim

As described above, Kuranouchi et al. have established a method of breeding low-temperature-tolerant lines through crossing and subsequent selection.

The cultivation of sweetpotato in cool regions is risky due to deterioration or the instability of root yield, but does have some merits. Noda et al. (2001) reported that the gelatinization temperature of sweetpotato starch is influenced by soil temperature during the outdoor cultivation period. They compared the starch from samples grown at 15°C with those grown at 33°C and found that the gelatinization temperature decreased greatly at 15°C. Nakamura et al. (2014) reported that sweetpotato cultivars containing starch with a lower gelatinization temperature have the potential for higher sweetness, as maltose generation can start earlier in heat-cooking. Takahama et al. (2013) reported that Brix (%) values of steamed sweetpotato roots were higher when

cultivated in Hokkaido prefecture, one of the coolest areas in Japan, compared to the roots harvested in warmer areas. On the other hand, they observed such problems as a slight darkening of flesh color and a decrease in dry matter content.

It is important to breed low-temperature-tolerant sweetpotato lines of high quality to expand the cultivation range of this crop. Therefore, it is essential that their roots provide good practical characteristics, such as appearance, flesh color, and cooking quality. Kuranouchi et al. are currently breeding new sweetpotato lines with low-temperature tolerance and with better practical characteristics.

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