ISSN 1341-9730

Sweetpotato Research Front

NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC) No.28, November 2012

Contents

Cover Story:	Recent Activity in Our Crop and Agribusiness Research Division	1
Research Paper:	Purification of Polyphenols from Sweetpotato (*Ipomoea batatas* L.) Leaves	2
	Transplantation Method of Half-Cut Tuber Seedlings in Sweetpotato Cultivar Murasakimasari	3
	New Radish and Sweetpotato Organic Upland Cropping System - Continuous Ridge Use	4
Research News:	Report of the Agri-Outlook-Forum, KARC Caravan: Development of New Sweetpotato Foliage Applications	5
Reader's Talk:	Sweetpotato Biotechnology in China	6

Recent Activity in Our Crop and Agribusiness Research Division

Ikuo Suda

Director of Crop and Agribusiness Research Division,
NARO Kyushu Okinawa Agricultural Research Center

Diverse varieties of field crops grow in the Kyushu-Okinawa region, including rice, wheat, barley, soybean, sweetpotato, potato, corn, buckwheat, Job's tears, sugarcane, strawberry, green and yellow vegetables, and fruit. In a research environment surrounded by field crops, our Crop and Agribusiness Research Division is advancing research in three fields: genetic improvement, food functionality, and agricultural economy.

In the genetic improvement field, soybean, sugarcane, buckwheat, and Job's tears are being genetically improved in the breeding programs for the Kyushu-Okinawa region. New varieties such as black-colored soybean “Kurodamaru” and “Kurosayaka”, spring-seeding buckwheat “Harunoibuki”, Job's tears “Akishizuku”, and high-sucrose sugarcane “Ni27” are being introduced in the region.

In the food functionality field, research covers clarifying metabolic regulatory functions of field crops effective for reducing risk of lifestyle-related disease, developing their evaluation technology, identifying functional ingredients, developing their analytical technology, and building a database of functional ingredients for the data obtained by a standardized analytical method. Field crops widely harvested in Japan are targeted. Data on the content of anthocyanin, the main functional ingredient of purple-fleshed sweetpotatoes (see SPORF No. 21, p4, and No. 23, p.5) is being accumulated. The anthocyanin content of black soybean, the contents of caffeoylquinic acid derivatives (see SPORF No. 23, p5), and lutein (see SPORF No. 21, p5) in sweetpotato leaves are similarly being investigated.

In the agricultural economy field, agricultural economists give farmers and the food industry useful information about the yield, quality, disease resistance, food functionality, and effective utilization technology of new varieties. This activity by agricultural economists is increasingly important for creating new agribusiness. Some consortia of agricultural producers and associates have been organized for new agribusiness opportunities. They include the successful commercialization of some new products using the black soybean variety “Kurodamaru”, purple-fleshed sweetpotatoes, and leaves of the sweetpotato variety “Suioh”. Thus, we conduct our research efficiently by cooperating with breeders, food scientists, and agricultural economists in the Division.

Research Paper

Purification of Polyphenols from Sweetpotato (*Ipomoea batatas* L.) Leaves

Rie Kurata[1] *and Makoto Yoshimoto*[2]

1. Upland Farming Research Division, NARO Kyushu Okinawa Agricultural Research Center
2. Kagoshima Women's Junior College

Sweetpotato leaves (*Ipomoea batatas* L.) contain a large amount of polyphenols that consist of caffeic acid (CA) and five types of caffeoylquinic acid (CQA) derivatives, mono- di- and triCQAs. Our previous study found many physiological functions, like DPPH-radical scavenging activity and antimutagenicity. In addition, we found that 3,4,5-triCQA had more effective antimutagenicity than mono- and diCQA derivatives. However, to our knowledge, 3,4,5-triCQA has only been isolated from *Securidaka longipedunculata, Tessaria integrifolia,* and sweetpotato leaf. 3,4,5-triCQA is a universal polyphenolic component in sweetpotato leaves. We therefore planned to establish a simple high-yield procedure for purifying 3,4,5-triCQA in order to investigate its physiological functions.

Figure 1 summarizes the procedure consisting of methanol extraction, water-hexane partition, MCI gel CHP20P (Mitsubishi Chemical Inc., Tokyo, Japan), and Sephadex LH-20 (GE Healthcare UK Ltd., Buckinghamshire, England) chromatography. All purification steps required fewer than three days, and the yield of purified 3,4,5-triCQA was calculated to exceed 76 mg from 100 g of leaf powder. Its recovery was estimated to be more than 66%, and its purity more than 98%. The 3,4,5-triCQA purification step established in this report had a much higher yield than reported for *Tessaria integrifolia* leaves (7.3 mg/100 g dry powder) and *Securidaka longipedunculata* roots (23.8 mg/100 g dry powder). We are now trying enzyme and cell-culture tests using 3,4,5-triCQA collected by this technique and studying the physiological functionality of 3,4,5-triCQA[1].

In addition, we slightly modified this purification method, adsorbed polyphenols in adsorption resin, and succeeded in collecting highly pure total polyphenols[2]. We hope that a product including these polyphenols (foods, cosmetics, pharmaceutical products, and biomaterials) is marketed in the near future because, sweetpotato polyphenols can be expected to have medical benefits.

(1) Kurata, R. *et al.*, Food Sci. Technol. Res., 17, 87-92(2011).

(2) Shimada,Y. *et al.*, Nippon Shokuhin Kagaku Kogaku Kaishi 57, 143-149(2010). [In Japanese with English summary]

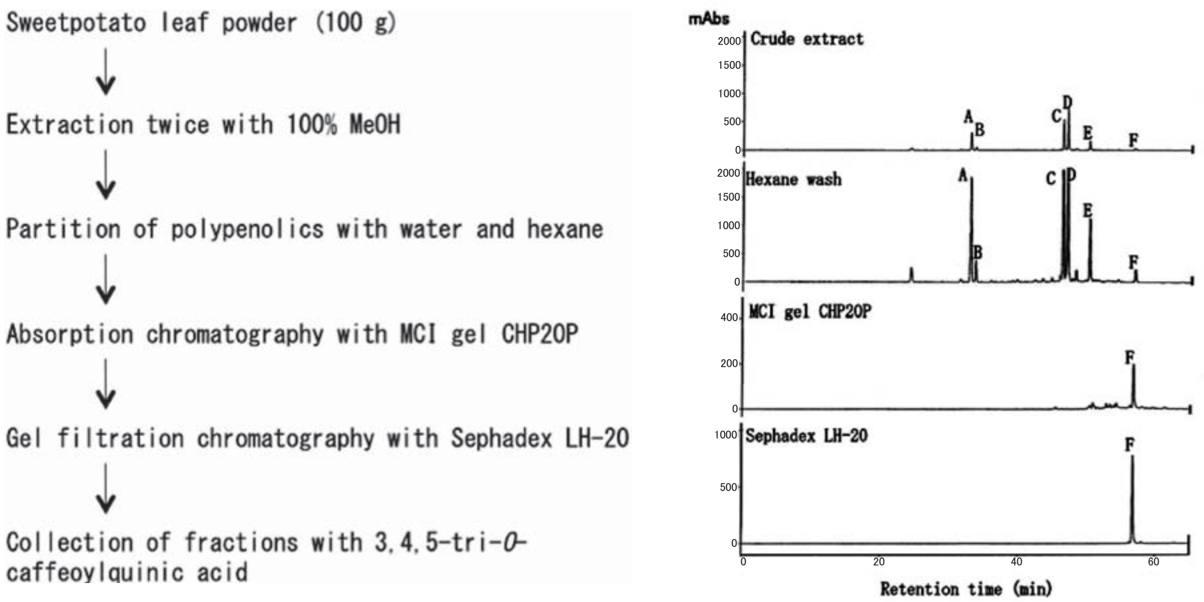

Fig. 1. Purification scheme for 3,4,5-tri-*O*-caffeoylquinic acid from sweetpotato leaves.
A: caffeic acid; B: 5-*O*-caffeoylquinic acid; C: 3,4-di-*O*-caffeoylquinic acid; D: 4,5-di-*O*-caffeoylquinic acid; E: 3,5-di-*O*-caffeoylquinic acid; F: 3,4,5-tri-*O*-caffeoylquinic acid.

Research Paper

Transplantation Method of Half-Cut Tuber Seedlings in Sweetpotato Cultivar Murasakimasari

Katsuki Adachi, Mitsuho Sugimoto, Masaaki Omine, Nobuyuki Sawamura, Takanori Ishii and Tooru Kobayashi

Upland Farming Research Division, NARO Kyushu Okinawa Agricultural Research Center

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is cultivated in Japan by a labor-intensive sprouted-vine planting method. In this study, we used the cultivar Murasakimasari to prepare seedlings from half-cut tubers planted in cell trays, referred to as cell-raised half-cut tuber seedlings. Here we propose a new sweetpotato cultivation method, *i.e.* transplantation of half-cut tuber seedlings in the cultivar Murasakimasari.

Seed tubers of sweetpotato cultivar Murasakimasari weighing 30 g to 100 g, were cut in half at a right angle to the long axis of the tuber, and planted in cell trays (cell size: 55 mm × 55 mm × height 62.5 mm, 50 cells in a 30 cm × 60 cm tray) with commercial soil mixes. The planted half-cut tubers in cell trays were incubated at 25°C under natural sunlight conditions in a glasshouse for three to four weeks to raise half-cut tuber seedlings (Figs. 1, 2). Half-cut tuber seedlings were transplanted (Fig. 3) on 28 March (tuber seedling transplanting TST1) or on 24 April (TST2). Whole seed tubers were directly planted on 26 March (direct planting (DP)), and conventional sprouted vines were planted on 30 April (vine planting (VP)) in an experiment field. The highest tuber yield was obtained from TST1, followed by TST2, VP, and DP in this order (Table 1). A rate of 3.0% (6.7%) of deformed tubers were observed in daughter tubers of the TST1 (TST2) group. A regional trial in a farmer's field revealed that the tuber yields and numbers of tubers per plant in TST exceeded those in VP (data not shown, see Adachi *et al.*, 2011). The results of the two field experiments suggest that transplantation of half-cut tuber seedlings provides enhanced yields over conventional sprouted-vine planting.

Reference: Adachi *et al.* Plant Prod. Sci. 14,291-297(2011).

Fig. 1. Photograph of cell-raised seedlings from half-cut tubers of cultivar Murasakimasari in a cell tray after 23 d incubation.

Fig. 2. Photograph of cell-raised seedlings from half-cut tubers removed from the cell tray after 24 d incubation. The distance between white lines is about 20 cm.

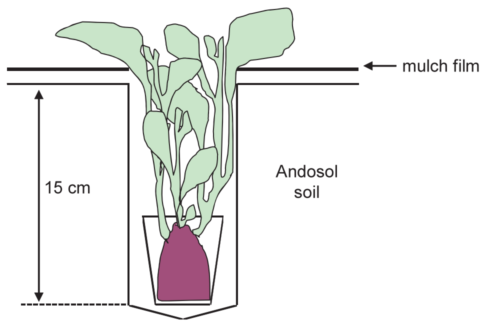

Fig. 3. Sketch of the transplantation method in the experiment field.

Table 1. Yield characteristics of TST1, TST2, DP, and VP in the experiment field.

Treatment	The date of transplanting / vine-planting	Shoot dry matter yield (g m^{-2})	Fresh daughter tuber yield (kg m^{-2})	Number of tubers per plant (no. $plant^{-1}$)
TST1	28 March 2008	295 ± 26	5.30 ± 0.26	12.6 ± 1.4
TST2	24 April 2008	246 ± 14	4.70 ± 0.26	9.8 ± 0.5
DP	26 March 2008	255 ± 21	4.13 ± 0.18	9.8 ± 1.3
VP	30 April 2008	238 ± 7	4.44 ± 0.27	8.5 ± 1.0

Values are average ± standard deviation of 4 replicates.
Daughter tubers more than 50 g fresh weight including deformed tubers are estimated.

Research Paper

New Radish and Sweetpotato Organic Upland Cropping System - Continuous Ridge Use

Hiroshi Niimi and Takayuki Suzuki

Upland Farming Research Division, NARO Kyushu Okinawa Agricultural Research Center

We have developed a new organic upland cropping system that does not require chemical fertilizer or agricultural chemicals. In one system, radish is cultivated by applying a condensed liquid made from sweetpotato distiller waste of *shochu* (a traditional Japanese liquor; Fig. 1). Ridges for radish are then used for sweetpotato (for *shochu*) cropping continuously, without tillage and fertilizer application (Fig. 2).

This continuous ridge use system is explained as follows. About 40% of the total nitrogen contained in condensed liquid distiller waste is easily decomposable and adsorbed by radish plants. The remaining nitrogen is available for sweetpotato plants because it is mineralized slowly at high soil temperatures in the summer. The condensed liquid is also rich in potassium necessary for radish and sweetpotato growth. Consequently, one application of condensed liquid is sufficient for growing both radishes and sweetpotatoes. Furthermore, the soil is tilled deeply and finely before radish cropping. The soil in and under the ridges thus remains soft after radish cropping, if the ridges are not stepped on. Continuous ridge use is possible for sweetpotato cropping from the viewpoints of both soil chemistry and soil physics.

Continuous ridge use makes continuous row use possible. In the system, "Tachi-ibuki" oats is cultivated in the rows. It covers the rows and inhibits weed germination through radish and sweetpotato cropping seasons without herbicide (Fig. 2).

The system was implemented at an organic certification field in Kyushu Okinawa Agricultural Research Center for three years, and a general cropping system using chemical fertilizer and agricultural chemicals with tilling before sweetpotato cropping was implemented in a contiguous field for comparison.

In the continuous ridge use system, the yields both of radishes and sweetpotatoes were comparable to those of the general cropping system (Fig. 3). Furthermore, there is little root-knot nematode injury in sweetpotatoes cultivated in the continuous ridge use system, and the injury index of the system was similar to that of the general cropping system after injecting soil fumigant (97% 1,3-dichloropropene) in each cropping (Fig. 4). We confirmed that the continuous ridge use system effectively reduced root-knot nematode injury in sweetpotatoes.

In many cases, the productivity of organic cropping systems is less than that of general cropping systems, and they have higher labor and materials costs. However, this continuous ridge use system facilitates high productivity and low cost. The system also recycles regional resources and contributes to environmental conservation.

Fig. 1. Application of condensed liquid made from sweetpotato *shochu* distiller waste

Mar. 16, 2010 ridge:radish row:oats　May 21, 2010 ridge:sweetpotato row:oats

Fig. 2. Continuous ridge use system

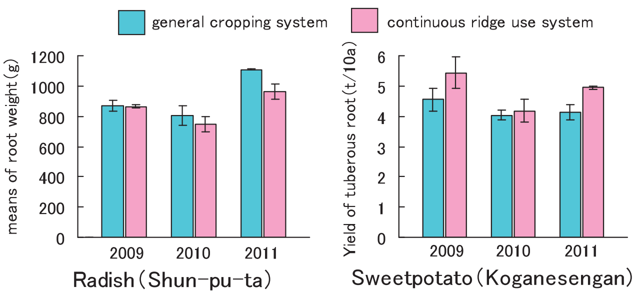

Fig. 3. Yield of radish and sweetpotato

Planting density of radish was 7143 (plants/10a) at continuous ridge use system in 2010 or 8333 at other plots.
Tuberous roots that weighed 30g or more were measured.

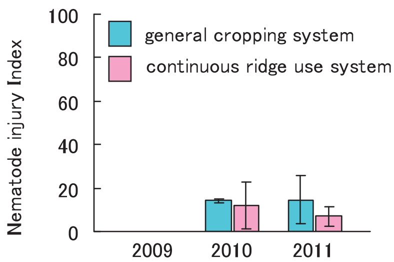

Fig. 4. Nematode injury index of sweetpotato (Koganesengan)

Injury of tuberous root was rated using an index ranging from 0 (no injury) to 4 (very heavy injury), and these index values were converted to a range of 0 -100. In 2009, injury was not shown at each plot.

Research News

Report of the Agri-Outlook-Forum, KARC Caravan: Development of New Sweetpotato Foliage Applications

Shigenori Okuno

Crop and Agribusiness Research Division, NARO Kyushu Okinawa Agricultural Research Center

"KARC Caravan", an Agri-Outlook-Forum sponsored by NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC), has been held several times a year since 2004. In the forum, executives of NARO/KARC visit field sites where the technology developed by NARO/KARC is used and exchange technical information with people there. The participants discuss how to solve problems regarding the technology of interest and promote its use more widely.

A KARC caravan with the theme of "Development of New Sweetpotato Foliage Applications" was held in Miyakonojo City, Miyazaki Prefecture, Kyushu, on June 6, 2012. The 42 participants included researchers of Kagoshima Prefectural Institute for Agricultural Development and NARO/KARC, and administrative officers of Miyakonojo City and Miyazaki Prefecture. The forum consisted of two sessions: (1) visit to the Shinsei Kosan Co., LTD. Plant where polyphenol extracts are manufactured from sweetpotato foliage (Fig. 1) and (2) visit greenhouses of Miyakonojo Primary, Japan Agricultural Cooperatives (JA Miyakonojo) for cultivating sweetpotato foliage (Fig. 2).

In the first session, the participants inspected the processes in the plant including washing sweetpotato foliage, extracting polyphenols, and concentrating the extract. The liquid polyphenol extracts manufactured in the plant are being examined for use in value-added products in manufacturing companies in food and other sectors.

In the second session, the participants looked around the greenhouses where sweetpotato foliage was growing. They discussed how to control insect pests and use harvesting machines.

The 2002 investigation in NARO/KARC revealed that the leaves of many sweetpotato cultivars contained higher amounts of total polyphenols than commercial vegetables and that sweetpotato leaves contained six polyphenols (caffeic acid, chlorogenic acid, 3,4-, 3,5-, and 4,5-di-*O*-caffeoylquinic acids, and 3,4,5-tri-*O*-caffeoylquinic acid). Numerous investigations worldwide have demonstrated that these compounds have many physiological functions including antioxidative activities. Based on these findings, use of sweetpotato foliage to supply polyphenols was thought to be a promising new application of the foliage.

NARO/KARC and Shinsei Kosan Co., LTD. have cooperatively investigated methods for effective preparation of polyphenols from sweetpotato foliage. During this cooperative work, the Shinsei plant was completed in 2011 and began full-scale operation in June 2012.

The plant operation is expected to contribute to increased consumption of sweetpotato foliage in southern Kyushu.

Fig. 1. Shinsei Kosan Co., LTD. Plant

Fig. 2. Greenhouse for growing sweetpotato foliage

Reader's Talk

Letter to the editor

Sweetpotato Biotechnology in China

Qingchang Liu

Professor & Associate Dean, College of Agriculture & Biotechnology, China Agricultural University

Sweetpotato is an important food and industrial material crop throughout the world. It is also an alternative source of bio-energy as a raw material for fuel production. China is the largest sweetpotato producer in the world, with an annual planting area of about four million hectares. Biotechnology offers great potential for improving disease, pest and stress resistance as well as nutritional quality of sweetpotato. In the past decades, great progress in sweetpotato biotechnology has been made in China.

An efficient system of embryogenic suspension cultures has been developed for a wide range of sweetpotato genotypes. So far, this embryogenic suspension culture system has been extended to more than 50 commercial cultivars of sweetpotato and gives very high frequencies of plant regeneration (over 90%). This regeneration system has great potential in somatic hybridization, in vitro selection of mutants, and genetic transformation of sweetpotato.

Somatic hybridization has been applied to overcome cross-incompatibility between sweetpotato and its wild relatives, and has generated useful interspecific somatic hybrids. Approximately 5,000 interspecific somatic hybrids have been produced from more than 20 sexually incompatible combinations by fusing protoplasts from embryogenic suspension cultures of sweetpotato and from petioles of the wild relatives using the polyethylene glycol (PEG) method. Several storage root-bearing, drought-tolerant, and high-quality interspecific somatic hybrids have been obtained. Genomic in situ hybridization (GISH) analysis has confirmed the presence of chromosomes from both parents and recombinant chromosomes in somatic hybrids.

Novel mutants have been obtained by cell-induced mutation and in vitro selection. It is suggested that 80 Gy gamma-rays are optimal for irradiation of embryogenic suspension cultures, and 30.0% PEG 6000 and 2.0% NaCl can be used as the optimal selection stress for in vitro selection of drought- and salt-tolerant mutants, respectively. Mutants exhibiting variations in root-skin color, root-flesh color, dry-matter content, and salt tolerance have been obtained. These mutants are non-chimeras and are applied in the cloning of important genes as well as in breeding of sweetpotato.

High importance has been attached to the cloning of agronomically important genes from sweetpotato in China. So far, more than 15 important genes associated with stem nematode resistance, salt tolerance, carotenoid biosynthesis, and anthocyanin biosynthesis have been isolated, and their functions have been identified.

Agrobacterium tumefaciens-mediated transformation has been standardized for important sweetpotato cultivars and has been used to produce transgenic plants resistant to diseases, stresses, and herbicides. Transgenic plants expressing *OC*I, *bar, LOS5, SOS, IbMIPS-1, SoBADH,* and *SBD2* genes have been achieved. *A. tumefaciens* strain EHA105 is strongly recommended for genetic transformation of sweetpotato embryogenic suspension cultures. It is also possible to successfully transform recalcitrant sweetpotato cultivars using this system.

The F_1 population of a cross between Xu 781, a line resistant to stem nematodes, with low yield and high starch content, and Xushu 18, which is susceptible to stem nematodes, has high yield and moderate starch content, is used to construct high-density molecular linkage maps of sweetpotato based on AFLP and EST-SSR markers. A total of 1962 (Xushu 18) and 1831 (Xu 781) markers were developed to construct two linkage maps. Both maps have the complete 90 linkage groups. Nine QTLs for dry-matter content are detected in Xushu 18 maps, explaining the variance from 11.4% to 45.6%. Twelve QTLs are detected in Xu 781 maps, explaining the variance from 7.9% to 40.2%. Several RAPD, AFLP, and SRAP markers linked to a stem nematode resistance gene have also been developed.

Editor's note

Genome sequencing was one of the hot topics in last Korea-China-Japan Sweetpotato workshop. Collaboration for sweetpotato genome sequencing by the three countries may begin in the near future (M.Y).

NARO

Sweetpotato Research Front(SPORF)

Published by the support from the

NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC)

Address:2421 Suya, Koshi, Kumamoto 861-1192, JAPAN

E-mail: sporf@ml.affrc.go.jp