ISSN 1341-9730

Sweetpotato Research Front

National Agricultural Research Center for Kyushu Okinawa Region (KONARC) No.18, August 2004

Contents

Three core technology and one target in KONARC

Osamu Yamakawa

Director General, National Agricultural Research Center for Kyushu Okinawa Region

In the National Agricultural Research Center for Kyushu Okinawa Region, we have departments responsible for upland farming, lowland farming, stockbreeding, and basic research on the agricultural environment. Many kinds of special crops are grown in the Kyushu and Okinawa region with its warm rainy weather. We are targeting these crops and performing research from three viewpoints (core technology), developing new cultivars, processing functional foods, and taking advantage of their environment-friendly utilization as biomass materials.

For instance, we breed sweetpotato, sugarcane, buckwheat, corn, soybean, strawberry, rice, wheat, barley, and grasses. Our research on physiological functions is famous worldwide. Rather than being limited to the basics, our research broadens to the development of new products in collaboration with companies. Research on anthocyanin (purple pigment) and pro-anthocyanin focuses on sweetpotato, soybean, rice, corn, and even tropical fruits. Also studied are the functions of the colorless caffeic-acid relatives, one of the polyphenolics in sweetpotato leaves.

Plenty of biomass exists in Kyushu and Okinawa because of the suitable weather conditions for plants. We are making great efforts to complete a model town where the biomass is wisely used, with no waste. In the first stage, we converted animal excretion into compost or energy; recently we have considered wood chips and food processing waste as biomass materials. Two current challenging projects are the classified extraction of beta-amylase, dietary fiber, and starch from sweetpotato; and the development of new seasoning from the wastes of sweetpotato liquor.

Our ultimate research goal is to establish in each local area a sustainable agricultural system with no waste. The sweetpotato is sure to be an important element of this system.

Research Paper

Purple sweetpotato anthocyanin reduces the intracellular hydrogen-peroxide (H_2O_2) level in bovine embryos caused by heat stress

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The rates of fertility and pregnancy of domestic animals in the Kyushu Okinawa region decrease in the summer. It is necessary to eliminate the factors causing this decrease in fertility. In the case of mammalian embryo development, heat stress decreases in the developmental ability both in vivo and in vitro. Recent studies have suggested a correlation between heat stress and oxidative stress in mammalian reproductive systems, including embryo development. Oxidative stress such as reactive oxygen species (ROS) is probably detrimental to preimplantation embryos. Therefore, a key to improving embryo development under heat-stressed conditions is reducing heat-sterss-induced oxidative stress. We have recently established a technique to detect hydrogen peroxide (H_2O_2) in individual preimplantation embryos with a ROS indicator. In this study, we use this technique to investigate the effect of heat stress on intracellular oxidative status, focusing on the generation of intracellular H_2O_2. Furthermore, we investigated whether purple sweetpotato anthocyanin, known as a natural strong free radical scavenger, prevents the excess generation of H_2O_2 in heat-stressed bovine preimplantation embryos.

Eight to 16 cell-stage embryos derived from in vitro maturation and fertilization were used for the experiment. Embryos cultured at 38.5 ℃ were assigned as the control group. Embryos exposed to 41.5 ℃ for 6 hr were assigned as the heat-stressed group. Embryos pre-cultured with 10 μg/ml purple sweetpotato anthocyanin at 38.5 ℃ followed by exposure to 41.5 ℃ for 6 hr were assigned as the heat stress+anthocyanin group. After exposure to heat stress, the levels of H_2O_2 of embryos in all three groups were measured with 2',7'-dichlorodihydrofluorescein diacetate (DCHFDA), a fluorescence ROS indicator. Fluorescence emissions of DCHFDA were measured by fluorescence microscopy and analyzed.

Heat stress significantly increased the level of H_2O_2 (Fig.1a, b; Fig.2). In contrast, adding anthocyanin to the culture medium significantly decreased the level of intracellular H_2O_2 of heat stressed embryos. These results indicate that a pre-culture of purple sweetpotato anthocyanin effectively scavenges the H_2O_2 in bovine embryos under heat-stress. Further studies will be necessary to clarify the relief from the detrimental effects of oxidative stress by mediating natural antioxidants in bovine embryos.

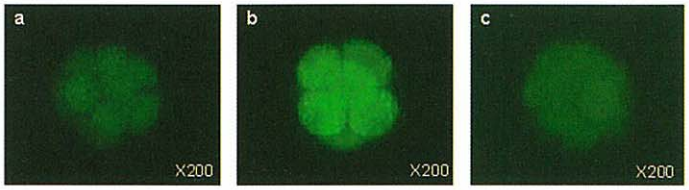

Fig. 1. Effect of anthocyanin on the generation of H_2O_2 in bovine embryos under heat stress
a: Control, b: Heat-stressed, c: Heat-stressed and pre-cultured with anthocyanin. Green indicates H_2O_2 fluorescence by DCHFDA.

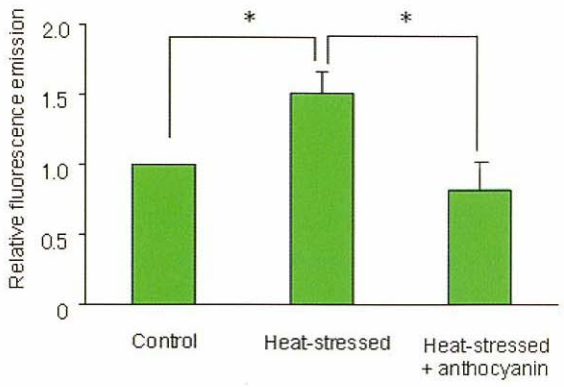

Fig. 2. Fluorescence emission of H_2O_2 in embryos by DCHFDA and the effect of purple sweetpotato anthocyanin
The results are shown as mean ± SEM by relative fluorescence emission vs. control. * Indicates significant difference ($p<0.05$).

Research Paper

Recent Progress in Characterization and Control of Virus Disease

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Laboratory of Molecular Plant Pathology

Sweetpotato feathery mottle virus (SPFMV) is common everywhere sweetpotatoes are grown in Japan. SPFMV is aphid-transmitted in a nonpersistent manner. Many strains have been identified in different parts of the world, and four strains (SPFMV-JC, -O, -S, and -T) have been recognized in Japan. SPFMV-JC, -O, and -T induce mild foliar symptoms but do not affect the yield of potato in most of the cultivars. A severe strain (SPFMV-S) is endemic in some producing areas and causes russet crack (Fig. 1), reducing yields drastically. These strains are readily distinguished directly from co-infected sweetpotato leaves by reverse transcription polymerase chain reaction (RT-PCR) and digestion by restriction enzymes (*Alu* I and *Hha* I).

Russet crack is endemic in some prefectures. Cross protection by mild strain (17-O) was developed by Oita Prefectural Agricultural Research Center. The isolate 17-O was selected by a series of mechanical transmission from naturally infected sweetpotato, which exhibited mild symptoms. Molecular characterization revealed that 17-O is closer to SPMFV-O than other strains. Several field experiments have been conducted by Oita Prefectural Agricultural Research Center, and the results suggested 17-O effectively reduces russet crack.

Recently, Sweetpotato virus G (SPVG) and Sweetpotato latent virus (SPLV) have been found in Japan. SPVG and SPLV were originally found in mainland China and Taiwan, respectively. Both viruses are morphologically similar to SPFMV. However, all attempts at aphid transmission have been unsuccessful. Serological tests have demonstrated that neither virus is related to SPFMV. The 3'-terminal nucleotide sequence (1.9 kb) of genomic RNA of SPVG was determined. Comparison with Chinese isolate revealed 93.5% identity at amino acid level (Fig. 2). The results suggested that Japanese SPVG is distinct from Chinese one. Japanese SPVG induces few foliar symptoms with no significant loss of potato yield.

The epidemic of SPLV is poorly understood in Japan. As the name suggests, many sweet potato cultivars infected by SPLV do not have obvious symptoms. Recently, we have cloned coat protein gene of the Japanese isolate of SPLV. Fusion protein with glutathione S-transferase (GST) was prepared and used to raise an antiserum in rabbits. Using the antiserum, SPLV antigen was readily detected by enzyme-linked immunosorbent assay (ELISA). The development SPLV detection by ELISA will facilitate our investigation on the SPLV epidemiology; hopefully, the occurrence and infection rate of SPLV among sweetpotato grown in Japan will be clarified soon.

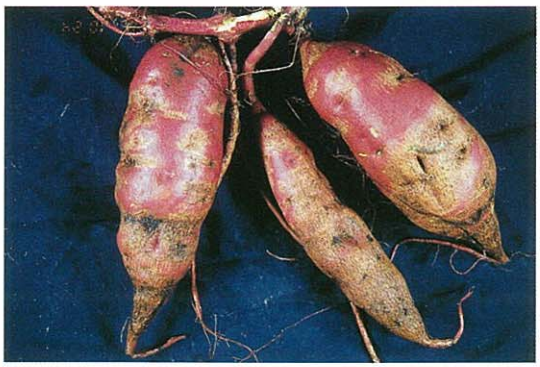

Fig. 1. External necrosis induced by the severe strain of sweetpotato feathery mottle virus (SPFMV-S) in a Kokei-type storage root. (Courtesy S. Yamasaki).

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  1 SAEEIYDAGKTGNTGRGRGRGNVPPPPPPPPGAPRTGDLPPAVQTGPLPPG  50
    |  ||  ||||  ||||||||||  |||||||||  |||  ||||||||||||
  1 STEETYDAGNTGNTGRGRGRGTVPPPPPPPPGTPRTTGLPPAVQTGPLPPG  50

 51 AASKPPIIEEIPQPESPRAKALREARGKAPATIPDSRGVDTSQIPSFTPG 100
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 51 AASKPPIIEEIPQPESPRSKALREAREKAPATVPDSRGVDTSQIPSFTPG 100

101 RDQTMTPTLQRTSTGARDRDVNAGTVGTFIVPRLQITHSKKRAPMANGRV 150
    |:|||||  ||||  |  ||||||||||||||  ||||||  ||||||||||||:
101 REQTMTPAPQRTSAGVRDRDVNAGTVGTFTVPRLQIAHSKKRAPMANGRI 150
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Fig. 2. Comparison (partial) of coat protein gene at amino acid level between Japanese (upper) and Chinese isolate of Sweetpotato virus G (SPVG).

Daichinoyume: New Sweetpotato Cultivar for Starch Production

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Introduction

"Daichinoyume" is a newly released cultivar with a high starch content and high yield; it was developed by the National Agricultural Research Center for Kyushu Okinawa Region. It was evaluated in the prefectural agricultural experiment stations as breeding line "Kyushu No.123," and officially registered as "Sweetpotato Norin No.59" by the Ministry of Agriculture, Forestry and Fisheries in 2003 for starch production.

Origin

"Daichinoyume" is a progeny from a cross between "Kyukei 117" and "Hi-starch" conducted at the Ibusuki branch of the station in 1990. Ninety-four seeds were sown in the nursery of the Sweetpotato Breeding Laboratory, and selected based on the field performance and starch content. Kyukei 117 has high storage ability and is resistant to root-knot nematode. Hi-starch has a high starch content and high yield.

Description

Daichinoyume, a prostrate plant type, has slightly good sprouting ability. The top leaves are light green. The mature leaves are light green and three-lobed with teeth. The vines are slightly thin with a medium internode length. Vines and vine node pigmentation of anthocyanin is absent. Storage roots are uniformly fusiform with white skin and light yellowish-white flesh. Its steamed root is not palatable, so Daichinoyume is not suitable for table use.

Performance

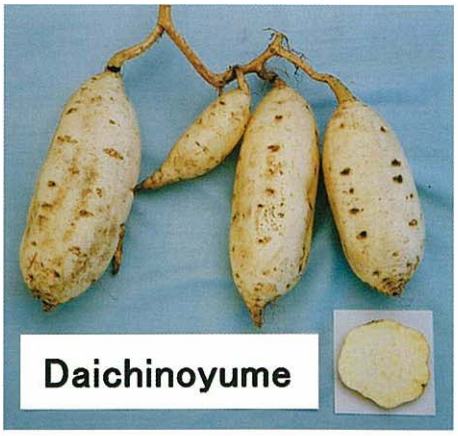
The root yield, starch content, and starch yield of Daichinoyume are higher than those of Koganesengan and are comparable to those of Konahomare.

Daichinoyume exhibits strong resistance to root-knot nematode, slightly strong resistance to the root-lesion nematode, and slightly weak resistance to black rot. The storage ability of Daichinoyume is superior to that of Koganesengan and Konahomare.

Yield and other traits of Daichinoyume in yield trial (1995-2002, standard harvesting)

Traits	Daichinoyume	Koganesengan	Konahomare
Root yield (t/ha)	27.8	25.0	27.7
Root size (g)	218	207	262
No. of roots per hill	3.4	3.3	2.8
Dry matter content (%)	39.4	36.2	39.4
Starch content (%)	26.9	24.8	27.3
Starch yield (t/ha)	7.5	6.2	7.6
Root-knot nematode resistance [1]	R	SS	SR
Root-lesion nematode resistance [1]	SR	SS	I
Black rot resistance [1]	SS	S	—
Storage ability of root in winter [2]	SH	SL	I

[1] R: Resistant. SR: Slightly Resistant. I: Intermediate. SS: Slightly Susceptible. S: Susceptible.
[2] SH: Slightly High. I: Intermediate. SL: Slightly Low.



Research Paper

The Laboratory of Plant Nematology (Nishigoshi)

Plant parasitic nematodes, such as the root-knot nematode or the root-lesion nematode, are a serious problem for sweetpotato cropping. They severely reduce yield and commercial value by producing cracks, necrotic spots, and/or constrictions on the storage root. Most sweetpotato growers inevitably depend on nematicides.

Our laboratory is now conducting research on races of southern root-knot nematode *(Meloidogyne incognita)*, the most dominant species in the sweetpotato fields of Southwest Japan. We have identified at least seven races of *M. incognita* in terms of pathogenicity to several sweetpotato cultivars. If the race of nematode is identified in the field that growers want to use, they can easily chose resistant sweetpotato cultivars to grow there. Moreover, crop rotation with resistant and susceptible cultivars could possibly reduce nematode density. We are now attempting such crop rotation on a trial basis. To facilitate the identification of the race, DNA analysis of the nematode is also underway.

Crop rotation test for reducing nematode density. A southern root-knot nematode *(Meloidogyne incognita)* is shown in the circle.

The Laboratory of Food Functions (Nishigoshi)

Previously called the Laboratory of Crop Quality, Storage and Processing, our laboratory was introduced in the SPORF, 8, p5 (1999). Due to the increasing demand for foods with health benefits, our study intensively focuses on the physiological functionality of the sweet potato and other agricultural products, and their utilization in foods and beverages in Japan. Our laboratory staff is comprised of analysts, biochemists, clinical chemists, and food processing specialists; all work in the Analytical Assessment Center for Producing Crops with High Quality, and the Physiological Function Assessment Center for Producing Healthy Food. Studies are proceeding with the mutual aid of each specialized field. A recent review of purple-fleshed sweetpotatoes containing anthocyanins appears in the JARQ, 37(3), 167-173 (2003) (http://ss.jircas.affrc.go.jp/engpage/jarq/37-3/37-03-04.pdf).

Staff of Laboratory of Food Functions

Reader's Talk

Letter to the editor

Chlorophyll Breakdown and Its Physiological Catabolites in Sweetpotato Leaves

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In the sweetpotatoes of Japan's South Kyushu region, russet crack disease, caused by a severe strain of Sweetpotato Feathery Mottle Virus (SPFMV-S), has prevailed since the 1970s. The disease has caused a devastating decline in yield and quality, with cracks and discoloration of the root tuber skin. As an effective disease-control measure, virus-free techniques using meristem culture have been developed and applied to the cultivation of various commercial crops such as strawberries and potatoes. In Miyazaki, meristem culture of the sweetpotato was begun in 1980 with a virus-free goal, resulting in the disappearance of the symptom and the elimination of the virus in the resulting tubers the next year. This cell-free system is beneficial not only to the sweetpotato farmers in Japan but also to those in developing countries in temperate regions and in the subtropical zones where prevailing viruses can destroy crops. The popularity of the Shochu has resulted in an output exceeding that of Sake, and the production of a high-quality Shochu is expected.

Quality degradation by infection of pathogenic fungi to the plant body along with physiological aging and senescence has been studied. Chl degradation results in cell death caused by external factors such as low or high temperature, or pathogen attack. However, the physiological function has long been poorly understood. Our group studies of heme catabolism in mammals have shown that bilirubin (BR) is an effective antioxidant. To investigate the physiological function of BR, anti-BR monoclonal antibodies, designated 24G7, were prepared. To clarify the physiological importance of the Chl catabolic system and/or its catabolites during the aging/senescence, we clarified that a BR-like compound was formed in the de-greening process of the sweetpotato (Ayamurasaki) leaves. The active oxygen radical scavenging activity of BR-like Chl catabolite (BRC) from the sweetpotato was examined. Moreover, to investigate their efficacy as an anti-cancer agent, induction of apoptosis was tested in human promyelocytic leukemia cells (HL-60), a valid model for testing anti-leukemic or general antitumoral compounds.

The results indicated high scavenging activities of BRC against superoxide anion and hydroxyl radicals, which are thought to be involved in aging and disease. These results suggest that BRC acts as a physiological antioxidant against oxidative stresses. Antioxidants, including N-acetyl-L-cysteine (NAC) and catalase, effectively block BRC-induced caspase-3 activation and DNA fragmentation. Thus, BRC may trigger an apoptotic cell-death program. Given BRC's anti-cancer function, these findings enhance our understanding of cancer chemoprevention of BRC in fruits, crops, and vegetable-based diets.

Announcements

As a result of the staff organization on June 1, 2004, we welcome K. Nonaka and H. Iwahori as new SPORF Coeditors, replacing H. Ikeda and Z. Sano.

Editor's note

I am a fledgling editorial staff member. It's a great pleasure to participate in SPORF as a coeditor. (H.I.)

Sweetpotato Research Front (SPORF)

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Published by the support from the National Agricultural Research Center for Kyushu Okinawa Region, NARO, Nishigoshi, Kumamoto 861-1192, JAPAN