Genetic Engineering of Amylose Content in Sweetpotato Starch by RNAi Technique

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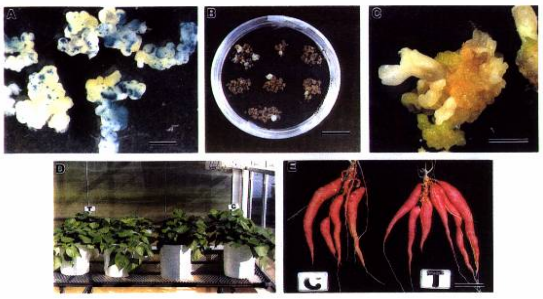
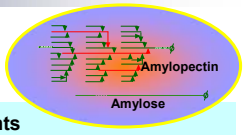
Agrobacterium– mediated transformation of sweetpotato using embryogenic callus



Aim of this study

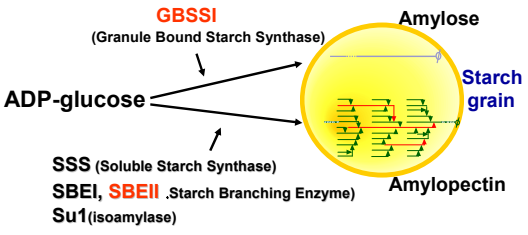


Production of sweet potato plants containing novel starch

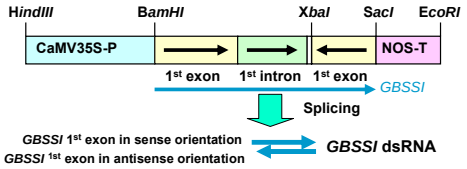
. The sweetpotato starch contains 10 – 20 % of amylose and 80 – 90 % of amylopectin.
. Amylose-amylopectin ratio is an important factor of textural properties of starch.
. A new sweetpotato variety having starch with new amylose content would be developed new dietary and industrial applications.

We use the RNAi (interference) technique for blocking the genes related to starch biosynthesis pathways.

Starch Biosynthesis and Key Enzymes



GBSSI RNAi construct

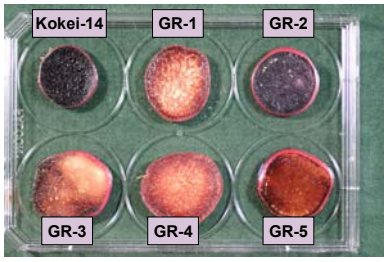


89 transgenic plants were regenerated and grew normally in a biohazard green house.
No difference between the transgenic and control plants.

After 4 month culture, normal roots were yielded.

Yield of tubers and starch of transgenic plants were almost same as the cotrol.

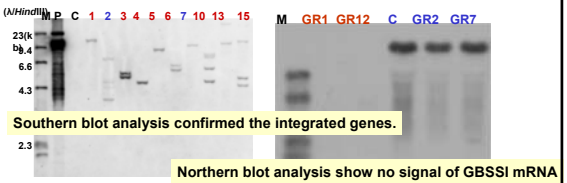
The sweet potato roots are cut into pieces and stained with Iodine solution



Non-transgenic, Kokei-14 and 5 transgenic lines (GR-1.5).
GR-2 shows dark-blue staining similar to Kokei-14, and others show red-brown staining.

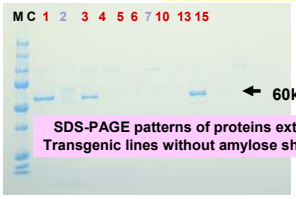
In total 38 transgenic plants, 28 lines (73.7%) were amylose-free

	No. lines	Dark Blue	Red Brown	. Amylose -free lines
Kokei 14 (Non-trg)	2	2	0	0
Transgenic plants Exp. 1	23	6	17	73.9
Transgenic plants Exp. 2	15	4	11	73.3

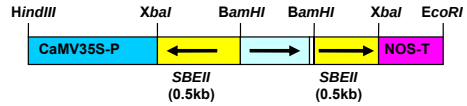


Southern blot analysis confirmed the integrated genes.

Northern blot analysis show no signal of GBSSI mRNA



SDS-PAGE patterns of proteins extracted from starch granules.
Transgenic lines without amylose show no band of 60 kD GBSSI protein.



pTIbSBEII-ASIS

pTIbSBEII-ASGS

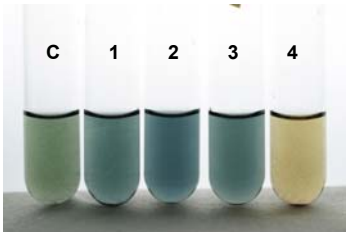
Spacer
GBSSI 1st intron (0.3kb)
or
Partial GUS (1.0kb)

Splicing

SBEII dsRNA

Construction of transformation vector of *SBEII* RNA interference to decrease the amylose content

We obtained total 8 transgenic lines (T).
The storage roots harvested after 4 months culture
in a biohazard green house



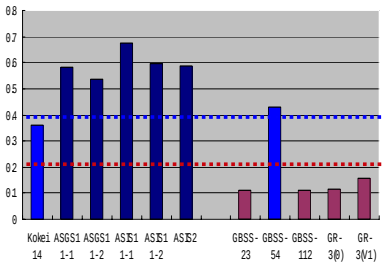
14.8 31.8 38.8 31.9 0 (%)
Amylose content

Iodine staining pattern of non-transgenic Kokei 14 (c),
transgenic lines with dsRNA of *SBEII* (1 - 3)
and a transgenic line with dsRNA of *GBSSI* (4)

Starch and amylose content of *SBEII* RNAi transgenic sweetpotato plants

Lines	Starch/30g of storage root (%)	Amylose content (%)
Kokei 14	5.8 (19.3)	10.3
ASIS-1	4.5 (15.0)	20.0
ASIS-2	4.9 (16.3)	23.4
ASGS-1	5.2 (17.3)	17.9
ASGS-2	4.7 (15.7)	23.3

Blue value of starch from transgenic lines



ASGS and ASIS lines transformed by dsRNA of *SBEII* gene
GBSS lines transformed by dsRNA of *GBSSI* gene

SUMMARY

The gene construct that encoded double stranded RNA of *GBSSI* first exon was introduced into the sweetpotato genome.

Starches from 73% of transgenic lines showed red-brown staining pattern by iodine staining and were confirmed to be amylose-free.

On the other hand, we also obtained the transgenic sweetpotato introduced with the gene construct encoding double strand RNA of *SBEII* fragment. Starches from the transgenic plants increased in amylose content by two-fold.

The results demonstrated that the amylose and amylopectin synthesis were inhibited by dsRNA of *GBSSI* and *SBEII* fragment in tubers of autohexaploid sweet potato plants, respectively.

These unique characteristics of starches may be useful for special products in the food industry.