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Yield-enhancing and tuber-downsizing effects of transplantation cultivation method of case-held tuber seedlings in the sweet potato cultivar Beniharuka

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ABSTRACT

We developed transplantation cultivation method of case-held tuber seedlings (CTS), which was derived from direct planting method of seed tubers, and applied this method to the sweet potato cultivar Beniharuka. A plastic case made of polypropylene was designed for cultivation of CTS. Seed tubers of cultivar Beniharuka in the range of 30–80 g were cut in half. The half-cut tubers were placed inside the plastic cases, and the cases were filled with a commercial soil mix. The case-held tubers were incubated under natural sunlight in a glass house. After 3–4 wk, the CTS were transplanted into a field. Mother tuber (seed tuber) enlargement was suppressed by the plastic confinement of the cases, and daughter tubers were formed above the case as vine-root-originated tubers. In the field experiments in 2012 and 2013, daughter tuber yields were increased 19% and 21% by case-held tuber seedling transplanting (CTST) over conventional vine-planting (VP), the number of daughter tubers per plant in CTST were 36 and 68% higher than in VP, and the mother tuber yields were limited to 2.1 and 4.3% of the total fresh yield of mother and daughter tubers, respectively in 2012 and 2013. Application of CTST method to cultivar Beniharuka enhanced tuber yield, increased the number of daughter tubers per plant, downsized daughter tubers compared to VP, and mother tuber enlargement was suppressed by case-holding. The CTST method is expected to produce more and smaller good in shape tubers of cultivar Beniharuka compared to VP.

Abbreviations: BTST bottled tuber seedling transplanting, CTS case-held tuber seedling, CTST case-held tuber seedling transplanting, DAP days after planting, FW fresh weight, TS tuber seedling, TST tuber seedling transplanting, VP vine planting

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Beniharuka; case-held tuber seedlings; mother tuber enlargement; prototype plastic case; sweet potato; transplant; yield

Introduction

In Japan, sweet potato plants (*Ipomoea batatas* (L.) Lam.) are cultivated using the conventional sprouted-vine planting method. Direct planting of seed tubers has several advantages over conventional vine planting (VP), including energetic growth of plants, high yield from widely spaced planting, and resistance to environmental stresses (Sakai, 1999), but has not been adopted widely. The direct planting method, which was developed in the 1950s and 1960s, uses whole seed tubers (Akita et al., 1962; Kodama, 1962). Kodama et al. (1957a, 1957b) investigated the growth of directly tuber-planted sweet potato and reported that, during the early stages of growth, both the shoots and underground parts (roots and tubers) of these plants grew more rapidly than those of plants cultivated by the VP method. They also showed that directly planted seed tubers in soil thickened into anomalous tubers. In

the direct tuber planting method of sweet potato, mother tuber enlargement and un-uniformity of above-ground sprouting in the field are practical problems preventing the widespread application of this cultivation technique.

We previously reported that the transplantation of tuber seedlings derived from half-cut tubers (i.e. TST) to achieve uniformity of above-ground sprouting in the field resulted in an enhanced yield compared to the conventional VP cultivation in the sweet potato cultivar Murasakimasari (Adachi et al., 2011). In contrast, in the cultivar Koganesengan, which is the most popular cultivar for sweet potato spirit production, the TST method resulted in mother tuber enlargement (Adachi et al., 2012), suggesting that the TST method was unsuitable for use with this cultivar. Next, we reported the succeeding transplantation method using bottled tuber seedlings (i.e. BTST) in the cultivar Koganesengan (Adachi et al., 2012). In the BTST method, mother tuber enlargement was physically

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suppressed by keeping the half-cut seed tubers in plastic bottles in the field, and daughter tubers were formed above the bottle. Then, we designed an improved plastic case according to the description of patent No. 5483091 in Japan (Adachi et al., 2014), and we conducted the transplantation of CTS (i.e. CTST) with the newly designed plastic case.

We first applied the CTST method to the cultivar Koganesengan and found that the shape of daughter tubers was sometimes longer than those by the conventional VP method (unpublished data). Furthermore, it was difficult to harvest these tubers using current sweet potato harvesters. Next, we applied the CTST method to the cultivar Beniharuka. The cultivar Beniharuka is for tabletop and processing uses and its use is increasing (Kai, 2009; Yoshinaga et al., 2010).

In this report, we compare the yield, number per plant, and size of daughter tubers in the CTST with those in conventional VP to clarify the effects of the CTST on the yield properties on the cultivar Beniharuka. The CTST method could suppress mother tuber enlargement and control uniformity of above-ground sprouting in the field on the transplanting day, hence the CTST may solve the practical problems of direct tuber planting method.

Materials and methods

Design of the plastic case prototype for the CTST method

The plastic case prototype for this study was made to the following specifications: 70 mm height, 40 mm × 40 mm top side square, 36.5 mm × 36.5 mm bottom side square, 1.0 mm thickness of side wall, 11.2 g weight, 91 mL inner volume, made with polypropylene, white in color, and containing a 9 mm ϕ drainage hole in the bottom.

2012 field experiment of the CTST Method

Seed tubers of the sweet potato cultivar Beniharuka, ranging in weight from 30 to 70 g, were cut in half at a right angle to the long axis of the tuber. The average fresh weight (FW) \pm standard deviation was 23.5 ± 6.3 g, ranging from 10.4 to 38.1 g. We used 98 half-cut tubers, and the cutting and planting took place on 5 April 2012. After small amounts of a commercial soil mix (Napura Soil Mixes, Yanmar Co., Ltd.) were poured into the case, the half-cut seed tubers were placed on the soil in the cases, and the soil mix was poured again on top to fill the cases. The case-held tubers were incubated under natural sunlight in a glass house at 25 °C, being watered daily or in a few days' intervals, depending on the weather and age of the seedlings. The CTS were transplanted with the cases into a field 4 wk later on 2 May 2012.

The 2012 experiment was conducted in an experimental field (latitude 31°45'N, longitude 131°01'E) at Miyakonojo Research Station, NARO Kyushu Okinawa Agricultural Research Center (NARO/KARC), Miyakonojo, Miyazaki Prefecture. The soil in the field was andosol, and the texture was loam (coarse sand 27.4%, fine sand 35.1%, silt 27.3%, and clay 11.2%). Manure was not applied on to the topsoil and lime was applied at a rate of 600 kg ha⁻¹. Chemical fertilizer was applied as follows: N 64 kg ha⁻¹, P₂O₅ 96 kg ha⁻¹, K₂O 160 kg ha⁻¹. The experimental area was 57.6 m² (4.8 m × 12 m). High ridges were set up with black plastic film mulch. The ridge distance was 100 cm, and the hill distance was 40 cm (planting density 25,000 hills ha⁻¹). Two different methods of planting were conducted: CTST and VP. For CTST, well-developed CTS were transplanted at a depth of 17–20 cm. For the VP, the sprouted vines (36.3 ± 3.6 cm long, with a stem length of 21.5 ± 3.4 cm, and their dry weight was 1.40 ± 0.47 g, sampled $n = 20$) were used. The vines were planted obliquely on 2 May. Each group was planted in duplicate. Subregions of 2 m × 1.6 m (two ridges with eight plants) in each plot were harvested to compare yield properties. In the subregions, the percentages of missing plants (vacant hills) were 0% in the CTST, and 10% in the VP group at harvest. The harvest date was 19 November 2012. The duration of cultivation was 201 d in both methods. Conventional weed and pest control measures were applied.

At harvest, we surveyed the yields to compare the following (1) daughter tuber yield (tubers of more than 50 g fresh weight only); (2) fresh weight of mother tubers; and (3) number and size (fresh weight) of daughter tubers per plant.

Preparation of CTS and tuber seedlings grown in a cell tray for the 2013 field experiment

Seed tubers of the cultivar Beniharuka, ranging from 50 to 80 g, were cut in half on 1 May 2013. The fresh weight range of the half-cut tubers, the average FW \pm standard deviation, and the number of half-cut tubers were 21.6–46.0 g, 33.0 ± 4.6 g, and 392, respectively. The half-cut tubers planted in the cases on 1 May were an equal mixture of top and bottom halves. The cases were filled with a commercial soil mix (Napura Soil Mixes). The tubers in the plastic cases were incubated at 25 °C in a glass house in natural sunlight. After 3 wk, well-developed CTS were transplanted into the field on 24 May.

To determine the effect of the case-holding, tuber seedlings (TS) were also generated in cell trays (Adachi et al., 2011). Seed tubers, ranging from 50 to 100 g in weight, were cut in half on 8 May 2013. The FW range of the half-cut tubers, average FW \pm standard deviation, and number of half-cut tubers were 24.0–53.5 g, 40.5 ± 5.4 g, and

350, respectively. Cut tubers were planted in plastic cell trays with a commercial soil mix (Napura Soil Mixes) on 8 May. Each 30 cm × 60 cm tray (Model 50AP-D, Tokan Kosan Co., Ltd.) contained 50 cells, and the cell size was 55 mm × 55 mm × height 62.5 mm. The cut tubers were incubated in trays, in a glass house, at 25 °C, in natural sunlight, to generate TS (Adachi et al., 2011). Plants were watered regularly, depending on the weather and the age of the seedlings. After 3 wk, well-developed TS were removed from the trays and transplanted on 28 May.

2013 Field Experiment of the CTST Method

The 2013 field experiment was conducted using an experimental field adjacent to that used in 2012. Manure was not applied. Lime and chemical fertilizer were applied at the same rates as described in the 2012 field experiment. The experimental area was 270 m² (19.8 m × 13.6 m). High ridges were set up with black plastic film mulch. The ridge distance was 80 cm, and hill distance was 40 cm (planting density 31,250 hills ha⁻¹). The experiment with three different methods of planting, i.e. CTST (planted on 24 May), TST (transplanting of tuber seedlings grown in a cell trays, 28 May), and VP (24 May), was conducted. For CTST and TST, well-developed seedlings were transplanted at a depth of approximately 20 cm and 15 cm, respectively. For VP, the sprouted vines (44.3 ± 5.2 cm long, with a stem length of 28.3 ± 3.3 cm, and vine dry weight of 3.01 ± 0.72 g, sampled *n* = 17) were used. The vines were planted obliquely. On 16 July (CTST and VP) and 17 July (TST), a growth survey (four plants in each plot) was conducted to compare growth among the three cultivation methods at 53 days after planting (DAP) (CTST and VP) and 50 DAP (TST). At harvest, subregions of 1.6 × 2 m (two ridges, 10 plants) in each plot were surveyed to compare yield properties. Each group was planted in four replicates. In the subregions, the percentages of missing plants (vacant hills) were 0% in CTST, TST, and VP. The harvest dates were 21 October for CTST and VP and 22 October for TST. The cultivation

durations were 150 d in both CTST and VP, and 147 d for TST. The same yield properties as described in the 2012 field experiment, along with the dry matter weight of shoots (leaves and stems), were determined in the 2013 field experiment.

Statistical analysis

The statistical analysis was conducted by both Analysis of Variance (ANOVA) and Tukey–Kramer HSD test.

Results

2012 field experiment of the CTST Method

As shown in Table 1, the fresh daughter tuber yield in CTST was 19% higher than in VP (significant at 5% level). In CTST, the mother tuber yield was 2.1% of the total fresh yield of mother and daughter tubers. The number of daughter tubers per plant in CTST was 36% higher than in VP (significant at 1% level).

As shown in Table 2, the average FW of daughter tubers in CTST was lower than that in VP, mainly because of the 36% increase in the number of daughter tubers per plant, despite the 19% yield increase. The percentage of the number of tubers weighing 50–100 g over the total number of tubers in CTST (21.3%) was much higher than in VP (7.7%), while the percentages of 700–1,000 g and 1,000 g ≤ tubers in CTST were lower than in VP.

Preparation of CTS and TS grown in a cell tray for the 2013 Field Experiment

The seedling emergence in CTS reached 40% after 10 d (Figure 1). After 23 d, an enough number of CTS for the field experiment were prepared (Figure 2) and transplanted into the field. On the other hand, the seedling emergence in TS grown in a cell tray reached approximately 50% after 10 d (Figure 1), and an enough number of TS grown in a cell tray was prepared after 20 d (Figure 3) and transplanted.

Table 1. Yield characteristics in the 2012 field experiment.

Treatment	Fresh daughter tuber yield (g m ⁻²)	Fresh mother tuber yield (g m ⁻²)	Total fresh tuber yield	Percentage of mother tuber yield	Number of daughter
			(mother and daughter tubers) (g m ⁻²)	over total fresh tuber yield (%)	tubers per plant (no. plant ⁻¹)
CTST	4673 ± 17	103 ± 4	4775 ± 13	2.1	5.9 ± 0.0
VP	3943 ± 123	-	3943 ± 123	-	4.3 ± 0.1
Ratio (CTST/VP)	1.19	-	1.21	-	1.36
ANOVA	*	-	*	-	**

Notes: Values are average ± standard deviation of two replicates. Daughter tubers with a fresh weight (FW) greater than 50 g are estimated. **, significant at 1% level in ANOVA analysis. *, significant at 5% level in ANOVA analysis.

Table 2. Average and number distribution of daughter tubers in FW in each treatment (CTST, VP) in the 2012 field experiment.

Treatment	Average of daughter	Maximum	Number distribution of daughter tubers in FW						
	tuber FW (g tuber ⁻¹ , ± SD)	tuber weight (g)	50–100 g	100–200 g	200–300 g	300–500 g	500–700 g	700–1000 g	1000 g ≤
CTST	317.4 ± 245.3	1368.1	21.3	16.0	21.3	21.3	12.8	4.3	3.2
VP	400.9 ± 293.8	1240.3	7.7	16.7	17.9	33.3	9.0	9.0	6.4

Notes: Daughter tubers with a FW greater than 50 g are estimated. Number of surveyed tubers and plants were 94 and 16 in CTST, and 78 and 18 in VP, respectively.

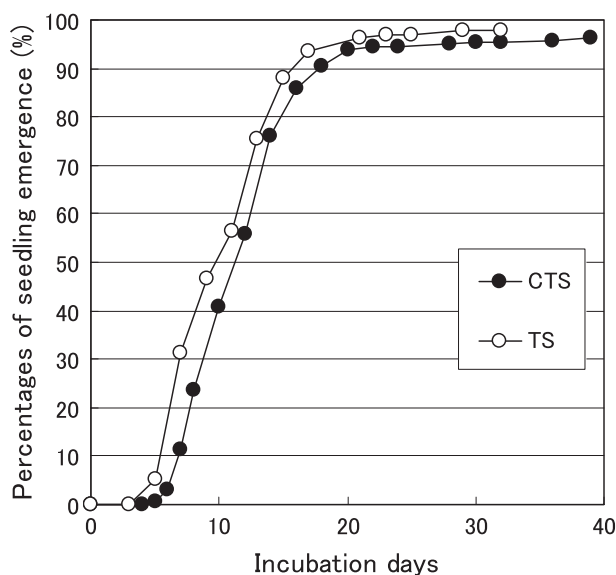


Figure 1. Percentages of seedlings that emerged above the soil surface in the plastic cases (CTS, case-held tuber seedlings) or in the cell tray (TS, tuber seedlings).



Figure 2. The case-held tuber seedlings of cultivar Beniharuka after 23 d incubation, and on the day of transplantation into the field. The vertical bar indicates 10 cm.

2013 Field Experiment of the CTST Method

In the growth survey at 53 DAP for CTST and VP, and 50 DAP for TST, the FW of daughter tubers (more than 5 mm



Figure 3. The tuber seedlings grown in a cell tray of cultivar Beniharuka after 20 d incubation, and on the day of transplanting into the field. The vertical bar indicates 10 cm.

φ tuber diameter) in CTST was significantly higher than that in TST (at 5% level) and tended to be higher than in VP (not significant) (Table 3). The number of daughter tubers (more than 5 mm φ diameter) in CTST tended to be higher than in VP (not significant). There were no significant differences among CTST, TST, and VP in shoot dry matter weight (leaves and stems) and maximum stem length per plant (Table 3).

To confirm the suppression of mother tuber enlargement by confinement in plastic cases, we set up a TST group without case-holding to compare to the CTST group. Using the CTST method, daughter tubers formed above the case, as vine-root-originated tubers (Figure 4). As shown in Table 4, the daughter tuber yield in CTST was 21% higher than that in VP (significant at 1% level), and the yield in TST was lower than that in CTST (significant at 5% level). The mother tuber yield in CTST was significantly lower than that in TST (significant at 5% level). The mother tuber yield in the CTST as a percentage of the total fresh yield of mother and daughter tubers was reduced to 4.3% compared to 8.2% in the TST without the case-holding. The number of daughter tubers per plant in CTST was 68% higher than that in VP (significant at 0.1% level). There were no significant differences in the shoot dry matter weight among CTST, TST, and VP (data not shown).

Table 3. Results of the growth survey conducted in the 2013 field experiment.

Treatment	Shoot dry matter weight		Daughter tuber FW	Number of daughter tubers per plant
	(leaves and stems)	Maximum stem length	(more than 5 mm ϕ tuber diameter)	(more than 5 mm ϕ tuber diameter)
	(g plant ⁻¹)	(cm)	(g plant ⁻¹)	(no. plant ⁻¹)
CTST	100.8 \pm 7.5	111 \pm 7	182.7 \pm 11.5 a	10.9 \pm 1.1
TST	108.9 \pm 4.4	114 \pm 16	114.6 \pm 40.2 b	9.4 \pm 2.7
VP	102.3 \pm 4.5	125 \pm 7	127.6 \pm 26.8 ab	7.7 \pm 2.3
ANOVA	ns	ns	*	ns

Notes: The growth surveys were conducted at 53 DAP for CTST and VP groups and at 50 DAP for TST group.

Values are average \pm standard deviation of four replicates.

Daughter tubers with a diameter more than 5 mm ϕ tuber are estimated.

*, significant at 5% level in ANOVA analysis.

ns, not significant in ANOVA analysis.

a, b, different small letter shows significance at 5% level in Tukey-Kramer HSD test.

In CTST, there was a higher daughter tuber yield and a lower mother tuber yield compared with TST. Since, in TST, mother tuber competes with daughter tuber for the allocation of photosynthates, the mother tuber enlargement in TST in the 2013 field experiment would have suppressed the daughter tuber yield.

The averages of daughter tuber FW (g per tuber) in the CTST and TST methods were lower than in VP (Table 5). The CTST and TST methods increased the numbers of 50–100 and 100–200 g FW tubers compared to those in VP (Table 5). However, the percentages of 300–400, 400–500, 500–700, and 700 g \leq tubers in VP were higher than those in CTST and TST (Table 5).



Figure 4. The stubble with daughter tubers and a case-held mother tuber in the CTST (A), and that in the conventional VP (B) at harvest. The vertical bars indicate 10 cm.

Discussion

We first developed the TST method (Adachi et al., 2011) for the cultivar Murasakimasari, one of the cultivars suitable for the direct tuber planting method (Sakai et al., 2007, 2008). Next, we developed the bottled tuber seedling transplantation (BTST) method (Adachi et al., 2012) for the cultivar Koganengan, which is not suitable for direct tuber planting or for TST cultivation method. The BTST method remained in a preliminary stage of development, because we used wide-mouthed plastic bottles designed for lab use, and made holes in the bottom for water drainage. The shape and size of the plastic container needed to be modified for optimal suppression of mother tuber yield (Adachi et al., 2012). Next, we designed the improve prototype of the plastic case, and developed the CTST cultivation method for the cultivar Beniharuka.

The shape, material, and strength of the newly designed plastic case prototype were suitable for use in this new cultivation method for the cultivar Beniharuka. Seed tubers of 30–80 g fitted inside after being cut in half. Ninety-eight cases (40 mm \times 40 mm in the top side square for each case) can be set in the regular tray (30 cm \times 60 cm) by placing 7 cases \times 14 lines. In the previous experiment, we compared the strength of four materials (polypropylene, polyethylene, styrene, and acrylonitrile–butadiene–styrene [ABS] resin) against the expanding pressure of mother tubers, and selected polypropylene because of good strength against cracks (data not shown). Continuous field experiments over several years showed that the plastic case is

Table 4. Yield characteristics in the 2013 field experiment.

Treatment	Fresh daughter tuber yield (g m ⁻²)	Fresh mother tuber yield (g m ⁻²)	Total fresh tuber yield (mother and daughter tubers) (g m ⁻²)	Percentage of mother tuber yield over total fresh tuber yield (%)	Number of daughter tubers per plant (no. plant ⁻¹)
CTST	3790 ± 147 A	169 ± 12	3959 ± 148 A	4.3	7.7 ± 0.6 A
Ratio (CTST/VP)	1.21	-	1.26	-	1.68
TST	3348 ± 123 AB	298 ± 67	3647 ± 143 AB	8.2	7.1 ± 0.8 A
Ratio (TST/VP)	1.07	-	1.16	-	1.56
VP	3143 ± 203 B	-	3143 ± 203 B	-	4.6 ± 0.3 B
ANOVA	**	*	***		***

Notes: The yield surveys were conducted at 150 DAP for CTST and VP groups and at 147 DAP for TST group.

Values are average ± standard deviation of four replicates.

Daughter tubers with a FW greater than 50 g are estimated.

***, significant at 0.1% level in ANOVA analysis.

** , significant at 1% level in ANOVA analysis.

* , significant at 5% level in ANOVA analysis.

A, B, different capital letter shows significance at 1% level in Tukey-Kramer HSD test.

Table 5. Average and number distribution of daughter tubers in FW in each treatment (CTST, TST, and VP) in the 2013 field experiment.

Treatment	Average of daughter tuber FW (g tuber ⁻¹ , ± SD)	Maximum tuber weight (g)	Number distribution of daughter tuber FW (% in number)						
			50–100 g	100–200 g	200–300 g	300–400 g	400–500 g	500–700 g	700 g ≤
CTST	157.9 ± 81.8	688.5	24.8	51.1	18.9	4.2	0.7	0.3	0
TST	150.9 ± 81.9	501.3	32.0	45.8	16.9	3.9	1.1	0.4	0
VP	219.9 ± 140.2	796.0	22.4	30.1	23.0	12.6	8.7	2.7	0.5

Notes: Daughter tubers with a FW greater than 50 g are estimated.

Number of surveyed tubers and plants were 307 and 40 in CTST, 284 and 40 in TST, and 183 and 40 in VP, respectively.

Table 6. Influence of cultivation methods on the shape of daughter tubers.

Cultivation year	Cultivation method	Percentage of each grade of daughter tubers (%)				Estimated tuber number (n)
		Grade 0	Grade 1	Grade 2	Grade 3	
2012	CTST	44.7	46.8	8.5	0	94
	VP	43.6	47.4	9.0	0	78
2013	CTST	63.0	35.6	1.5	0	135
	TST	64.1	28.2	6.8	0.9	117
	VP	46.4	46.4	7.1	0	84

Notes: Grades of daughter tubers were classified on the basis of the external appearances (e.g., long, round, bent, cracked, or constricted).

Grade 0: normal shape (good shape); Grade 1: slightly irregular shape; Grade 2: obviously irregular shape; and Grade 3: remarkably irregular shape (coiled).

Number of surveyed plants were 16 in CTST and 18 in VP in 2012, and 16 in each CTST, TST, and VP in 2013.

reusable for at least two-time cultivations in two years; however, after two years, some of the cases may be deformed by inner expanding pressure of the mother tuber or outer pressure by surrounding daughter tubers, and may have to be discarded.

The 2013 field experiment investigated the CTST method of the cultivar Beniharuka. As shown in Table 3, during the growth survey performed at 53 DAP, the fresh weight and the number per plant of the daughter tubers (more than 5 mm ϕ tuber diameter) in CTST were tended to be higher than that in VP. The increased daughter tuber weight and more number of daughter tubers per plant in the early growth stage may link with the increased yield and more number of daughter tubers at harvest.

Enlargement of mother tubers in the CTST at harvest was suppressed to 4.3% of the total fresh yield of tubers by case confinement. Furthermore, the daughter tuber yield in CTST increased by 21% compared to the conventional VP. On the other hand, in TST, in which mother tuber enlargement was not suppressed by case-holding, a 7% increase in daughter tuber yield was observed compared to VP. The mother tuber yield in TST was significantly higher than that observed in CTST. In addition, the CTST method resulted in a clear increase in the number of daughter tubers per plant compared to VP. Application of CTST to the cultivar Beniharuka is desirable for yield enhancement as well as suppression of mother tuber enlargement.

Furthermore, as shown in Tables 1 and 4, the CTST method clearly increased the number of daughter tubers per plant in the cultivar Beniharuka, resulting in smaller daughter tuber production compared to VP (Tables 2 and 5). The sweet potato cultivar Himeayaka with compact size storage root (for smaller sized tuber production) was released (Ohara-Takada et al., 2011), and registered in 2011 for tabletop use. When farmers are required to produce the smaller sized tubers of the cultivar Beniharuka, the CTST method may be suitable for this purpose. Beniharuka is superior in shape as the bred property (Kai, 2009), and the shape of daughter tubers under CTST cultivation conditions was normal and not inferior to that in the conventional VP in the present experiments (Table 6).

The field experiments in 2012 and 2013 suggest that the CTST method is effective for the cultivar Beniharuka and results in an approximately 20% increased yield of daughter tubers. The plastic case prototype is suitable for a new cultivation method in the cultivar. The plastic case prototype was acceptably strong for a suitable container against expanding pressure of mother tubers for this cultivar. However, in this new cultivation method, properties of the cultivar Beniharuka yield are affected by the date of planting, duration of cultivation, plant density, and weather conditions. Hence, further experiments are required to establish the practical technology to utilize the CTST method for yield enhancement. Furthermore, in CTST method, the plastic cases have to be collected at harvest and can be reused the following year, although some may have to be discarded because of deformation. Although it is necessary to estimate the labor and cost associated with the CTST method including the collection and reuse of plastic cases, the 20% yield enhancement is a considerable advantage over the conventional VP method.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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*In Japanese with English summary.

**In Japanese with English title.

***In Japanese, and the title was translated by the present authors.