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Field Trials of Tissue Culture Taro (*Colocasia esculenta* (L.) Schott) in the Northern Mariana Islands

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ABSTRACT

The Commonwealth of the Northern Mariana Islands consists of a group of islands in the western pacific. Taro (*Colocasia esculenta* (L.) Schott) is one of the important subsistence food crops grown in the Northern Marianas. The quality and production of taro crop has been severely affected in recent years by the introduction of major diseases and pests impacting taro production. The downturn in the islands' economy, natural disasters such as droughts and typhoons, and paucity of quality planting material have also had considerable constraints on taro production in the Commonwealth. The Cooperative Research, Extension and Education Service Department of the Northern Marianas College introduced a tissue culture program for the *in-vitro* multiplication and propagation of quality planting material of taro. New varieties of taro produced *in-vitro* were introduced from the regional certified institution and evaluated for tolerance to insect pests and diseases, plant growth, corm quality, high yield, taste and superior agronomic characters. Twenty-three varieties of taro propagated through tissue culture were tested at the Agriculture Experiment Station. Field trials and taste testing of new varieties were successfully conducted throughout the islands of Saipan, Rota and Tinian. Results indicated that new varieties performed well in local soil and climatic conditions of the Northern Mariana Islands. Japanese varieties (CA/JP-01, 06 and 09) rated excellent for taste, quality of corms, easy cooking, plant growth and yield comparable to other varieties. The selected varieties of taro were identified as the economically and culturally important ones that have been tested for superior characteristics in the region.

Keywords: Pacific, production, root crops, tissue culture, variety trials

Abbreviations: **CePCT**, Center for Pacific Crops and Trees; **CNMI**, Commonwealth of the Northern Mariana Islands; **CREES**, Cooperative Research, Extension and Education Service; **CSREES**, Cooperative State Research, Extension and Education Service; **IAA**, indole-3-acetic acid; **MS**, Murashige and Skoog; **NMC**, Northern Marianas College; **SPC**, Secretariat of the Pacific Community; **TC**, tissue culture; **USDA**, United States Department of Agriculture

INTRODUCTION

The Commonwealth of the Northern Mariana Islands (CNMI) is a chain of forested volcanic islands of the Marianas archipelago located in the Western Pacific. Subsistence farming, fruits, vegetables and root crops production are the main agricultural enterprises (USDOI-OLA 1999; Nandwani *et al*. 2007). The climate is characterized by high temperatures, heavy rainfall, and high humidity. The rainfall in the Sabana conservation area on the island of Rota is estimated to be as high as 260 cm annually, where taro is grown for commercial production. Most of the taro is planted in the Sabana area, and very few farmers grow taro on dry land. Taro (*Colocasia esculenta* (L.) Schott) is an economically important food crop and highly valued for cultural, dietary, and agriculture production in the CNMI. Nutritionally, taro is rich in fiber, calcium, potassium, iron, vitamin A, vitamin B1, vitamin B2, and vitamin C. The corms are baked, roasted or boiled, and leaves represent a significant source of vitamins, especially folic acid (Awasthi and Singh 2000; Cho *et al*. 2007). In addition to being an important traditional food crop, taro is a significant export commodity. The island of Rota is the major producer of taro, where commercial farming of taro is in practice. It is known for its traditional use in food preparations for special occasions. There are approx. a dozen cultivars of a taro, cultivated locally. Taro is the second most widely produced root crop in the CNMI after sweet potato. The total area under taro production in the CNMI in 2007 was 61 acres after sweet potato (71 acres) (Clark 2009). The total production of taro and quantity increased for five years during the period 2002 to 2007 (**Table 1**). Annually, over 50% of the taro produced on the island of Rota was exported to the neighboring island of Guam (approx. 3,000 lb/week). Little is consumed locally due to low population on the island and the balance was exported to Saipan both for consumption as well as for traditional cultural ceremonies (Nakamoto *et al*. 1996).

The objectives of the taro variety trials program in the Northern Marianas are to introduce tissue-cultured plants of

Table 1 Production of taro in the Northern Marianas for five years during 2007 and 2002.

	Year		Islands					
			Rota		Saipan		Tinian	
	2007	2002	2007	2002	2007	2002	2007	2002
No. of farms	69	60	30	38	37	21	2	1
Area (Acres)	61	48	53	44	(D)	(D)	(D)	(D)
Production (Tones)	100.514	72.536	79.548	67.177	(D)	(D)	(D)	(D)

D; Detail may not add to total due to rounding
Source: Agriculture Census 2007, Department of Commerce, CNMI

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new varieties and to select high-yielding, drought, disease- and insect pest-tolerant varieties with good corm quality through selection and field evaluation. The seedlings derived from tissue culture were evaluated in a series of trials and on-farm trials in all the three main islands of Northern Marianas.

Traditional planting methods using corm pieces and shoot apex for taro are time consuming and labor intensive. If the parent corm is infected with diseases, they are transmissible to the next generation, in spite of the application of fungicide or insecticide (Morishita 1988). Tissue culture is considered the most potential system to achieve the goals of producing quality and disease-free planting material (Taylor 1998; Chand *et. al*. 1999). High production costs due to increased labor and management, spread of soil-born diseases and pests, limited genetic diversity and shortage of quality planting material are the major constraints of taro production in the CNMI (Nelson and Geraledine 2000). Among the diseases, *Dasheen mosaic virus*, *Phytophthora* leaf blight, Southern blight or stem rot, leaf mold, leaf spot, soft rot of tubers are the common in the CNMI (Quebral *et al*. 1989; Matalog 1992; Kohler *et al*. 1997). To meet the challenges in taro cultivation, Northern Marianas College's Cooperative Research, Extension and Education Service (NMC-CREES) initiated a tissue culture program on taro for the production of disease-free and quality propagating materials. After screening accessions on the Agriculture Experiment Station and on-farm trials, selected lines propagated through tissue culture were distributed to the local farming community. This paper reports the results of field evaluations of tissue cultured taro accessions in the local soil and climatic conditions of the CNMI.

MATERIALS AND METHODS

Approach

Elite cultivars of taro were imported, while evaluation and selection of promising varieties was done under local conditions. The tissue culture laboratory of the NMC-CREES imported several pathogen-tested (virus-indexed) tissue culture taro cultivars into the CNMI for screening and evaluation. A total of 23 taro accessions were tested in the CNMI in 2007, 2008 and 2009 (**Table 2**). Parents or the names of pure line of each accession and their origin are listed in **Table 3**. Tissue culture plants of 23 accessions of taro were imported from the Centre for Pacific Crops and Trees (CePCT), Secretariat of the Pacific Community (SPC), Fiji. Upon receipt of package, plantlets were transferred to plastic begs with commercial potting mix (ACE Hardware, Saipan) and kept in the nursery (57% shade) for 2-3 weeks for hardening and acclimatization. Well hardened plants were transferred to the field at the As Perdido Agriculture Experiment Station, Saipan. A randomized plot selected, cleared of shrubs, grasses and tilled. Plantlets transferred in the field with 45-50 cms spacing within the plants and 60 cms apart in rows. Plants were rain-fed or irrigated when required and fertilized with commercial grade fertilizer (NPK, 16: 16: 16) at the time of planting. Plants were monitored periodically and data were collected on several specific characters evaluated in each trial. They included plant characters such as plant type, petiole color, plant vigor, yield, eating quality of corms, insect pest and diseases, maturity etc. At least six replicates (plants) of each variety were planted in the trials. Three trials were conducted in Saipan and one each on the islands of Rota and Tinian. CREES Crop Protection staff conducted scouting of crop for insect pest infestation and diseases damage. Field trips to all the three island locations were undertaken to the study areas, photos of plants and plant parts were taken and documented.

Taste testing

New varieties were evaluated for eating quality by taste testing (sensory evaluation) after harvesting. Corms of all 23 varieties were harvested, cleaned, washed and prepared for the taste testing evaluation. Corms were boiled, peeled off and cut into small pieces. All the varieties were displayed in foil trays and labeled. Three taste testing evaluations were conducted; two on the island of Saipan in January 2008 and January 2009 and one on the island of Rota in August 2008. A survey form was prepared for the participants to fill out during the taste testing. Participants of ages from 18 to 65 years (male and female both) including youths, students, faculty, research and extension agents, farmers, in the community were participated in the sensory evaluation. At least 60 participants evaluated the cooked corms in the event held in the island of Saipan in January 2008 and in the island of Rota in August 2008. Approximately 35 participants – mainly students, faculty, staff and administrators – participated in the event held on the NMC campus in January 2009 in Saipan. Data were collected on taste, texture and acceptability of corms through survey form and interviewing the people.

Micropropagation

Shoot-tip explants (apical and axillary buds) were collected from the TC taro plants grown at the As Perdido Experiment Station. Explants were washed in running tap water and shoot tips were excised from lateral cormels to about 1 cm. The explants were dipped in 70% ethanol for 30 sec. They were sterilized with 2% sodium hypochlorite solution and washed several times with sterile distilled water to remove the sterilizing agent. Shoot tips (meristematic dome with one or two leaf primordia) 0.3-0.5 mm in size excised from these buds were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) containing IAA (0.1 mg/l) and Kinetin (1.0 mg/l) and sucrose (30 g/l) at 23°C under a 12-h photoperiod (2500 lux). Growth regulators were obtained from Phytotechnology Laboratory, USA. Explants were subculture on fresh medium in 4-5 weeks for multiplication. Well-elongated shoots were cultured on basal MS medium for root induction. Regenerated plantlets were transferred to pots with potting soil (ACE Hardware, Saipan) for hardening. Well-hardened plantlets were transferred to the field after 2-3 weeks.

RESULTS

Varieties evaluated and selected, first under local conditions in on-station trials in Saipan, the main island in the CNMI (**Fig. 1**) and then trials conducted in islands of Rota and Tinian. Selected varieties were cultured in a tissue culture laboratory and plantlets sent to the two islands. Three variety trials of taro were conducted in Saipan in 2007, 2008 and 2009. A preliminary trial was conducted on the islands of Rota and Tinian in 2008. The main objective of the variety trials program was to select high-yielding, disease- and pest-tolerant cultivars with good corm quality characteristics acceptable to farmers and consumers in the CNMI.

Table 2 On farm and on-station variety trials of taro at the As Perdido Agriculture Experiment Station in the CNMI.

Trial	Island	Total № of accessions	Planting	Harvesting	Farm location	Taste testing
1st	Saipan	23	April 2007	December 2007	Agriculture Experiment Station, As Perdido	January 2008 As Perdido Farm
	Rota	23	January 2008	August 2008	1. Sabana Conservation Area 2. Sinapalo village	August 2008 NMC-CREES
	Tinian	23	February 2008	August 2008	Experimental plot, NMC-CREES, Tinian	-
2nd	Saipan	23	Feb 2008	August 2008	Agriculture Experiment Station, As Perdido	January 2009 NMC-CREES
3rd	Saipan	23	September 2008	April 2009	Agriculture Experiment Station, As Perdido	-

Table 3 Results of field evaluation of new varieties of tissue culture taro conducted at the As Perdido Experiment Station in Saipan from three trials conducted in 2007, 2008 and 2009.

Taro	RGC Acc No.	Variety	Origin	Avg. fresh weight of corm (kg)	Yield/ plant (kg)	Color of leaf/petiole	Inset pests and diseases	Plant growth[1] and corm size[2]
Colocasia esculenta	CA/JP-01	Tsuronoko	Japan	0.1	0.75	Green/green	Taro hornworm, browning in the leaves	Short and small,
var.	CA/JP-06	Akame	Japan	0.35	1	Dark green/purple-green	Taro hornworm	rounded
antiquorum	CA/JP-08	Takenoko-imo	Japan	0.1	0.65	Green/light purple-dark green	Red spider mites	
C. esculenta var.	BL/SM/04	C2-132	Samoa	0.95	0.95	Green	Taro hornworm, aphids, red-banded thrips	Giant and big
esculenta	BL/SM/12	C2-234A	Samoa	0.85	0.85	Green/red purple at the leaf base	Leaf spots, mealybugs, ants	
	BL/SM/22	C2-227	Samoa	0.6	0.6	Green/dark green	Plant hopper, taro hornworm, browning of leaves	
	BL/SM/31	C3-7	Samoa	0.7	0.7	Green/red purple at the leaf base	NA	
	BL/SM/47	C3-167A	Samoa	1	1	Green/red purple at the leaf base	Plant hopper, Chinese rose beetle	
	TAN/MAL/07	MAL 136	Malaysia	0.3	0.45	Green/dark green-purple	NA	Medium and
	TAN/MAL/08	MAL 141	Malaysia	0.5	0.5	Green/red purple at the leaf base	NA	moderate, cylindrical
	TAN/MAL/12	MAL 148	Malaysia	0.85	0.85	Green/red purple at the leaf base	Red spider mites, plant hoppers	
	TAN/IND/13	IND 245	Indonesia	0.5	0.5	Dark green/green (adaxial surface) and purple (abaxial surface)	Plants hoppers, scale, ants	Tall and moderate, rounded
	TAN/IND/19	IND 399	Indonesia	0.5	0.5	Green/red purple at the leaf base	NA	
	TAN/IND/ 20	IND 400	Indonesia	0.35	0.35	Green/light purple	Taro hornworm, red-banded thrips, ants	
	TAN/IND/23	IND 472	Indonesia	0.3	0.35	Green/light purple	Taro hornworm	
	TAN/PHL/02	PH 038	Philippines	0.25	0.5	Green/red-purple	Taro hornworm, aphids	Medium and
	TAN/PHL/05	PH 055	Philippines	0.25	0.3	Green/red-purple	Leaf spots, aphids	moderate,
	TAN/PHL/15	PH 164	Philippines	0.4	0.4	Dark green/red purple	Aphids, ants	mostly rounded
	BL/PNG/03	C2-E3	PNG	0.45	0.75	Dark green/green-pink at leaf base	Chinese rose beetle, aphids, red spider mites, negro bug, taro hornworm	Giant and big corms
	BL/PNG/06	C2-E8	PNG	0.55	0.55	Green/purple	Chinese rose beetle, aphids	
	BL/PNG/08	C2-E11	PNG	0.75	0.75	Green/purple	Taro hornworm, leaf spots and ants	
	BL/HW/12	MH 24	Hawaii	0.65	0.65	Green/dark green-purple	Taro hornworm and ants	Medium and
	BL/HW/26	MH 40	Hawaii	0.9	0.9	Green/green	Taro hornworm, leaf spots	small, rounded

[1]; Short-12", Medium-24", Tall 36", Giant- 48" (30, 60, 90 and 120 cm, respectively)
[2]; Small - 2.0-2.5", Moderate- 3-4.5", Big- 5-6" (5-6, 7.5-11 and 12.5-15 cm, respectively)

Fig. 1 Taro variety trial experimental plot at the As Perdido Agriculture Experiment Station, Saipan.

Field evaluation

Names and country of origin of imported taro and results obtained from the variety trials and evaluations conducted in Saipan are summarized in **Table 3**. All the varieties survived under field conditions and produced corms in local soil and climatic conditions on three islands of the CNMI, Saipan, Rota and Tinian. The average period from planting to harvesting of crop was approx. 7 months. Higher yield was obtained from the trials conducted in wet (rainy) season compared to dry season (February-May). Hawaiian (BL/HW/26), Japanese (CA/JP-06) and Samoan (BL/SM/04, BL/SM/47) varieties produced high yields (0.9 to 1.0 kg/plant), comparable to BL/SM/12, Malaysian (TAN/MAL/12), Papua New Guinean (BL/PNG/03 and BL/PNG/08) varieties, which produced moderately high yields (0.75-0.85 kg/plant). Philippines (TAN/PHL/02, 05 and 15) and Indonesian (TAN/IND/13 and 19) varieties were among the lowest yielding varieties (0.3 to 0.5 kg/plant). BL/PNG/03, TAN/MAL/12 and BL/SM/31 varieties observed high yields in the dry season with dry matter and tolerance to drought. In the on-farm trials, BL/SM/04, TAN/MAL/07 and TAN/MAL/08 varieties yielded moderately high, however, rated lower in taste tests by the community due to a prickly sen-

Fig. 2 Corms of one of the most favorable Japanese taro variety.

Fig. 3 Tissue cultures of taro at NMC-CREES growth room.

sation. In the on-farm trials conducted in 2007, 2008 and 2009, the Indonesian varieties (TAN/IND/13, 19, 20 and 23) yielded slightly lower than Malaysian varieties, but were moderately favorable in the taste test due to mostly dry and hard matter. Japanese varieties were short (30 cm) plants with small corms up to 5 cm in size (**Fig. 2**) and round shape compared to Hawaiian varieties with slightly taller plants (45 cm) and bigger corm size (6 cm). Samoan varieties were giant (120 cm) and yielded big corms comparable to Papua New Guinean varieties. Malaysian varieties yielded medium size (10 cm) corms with cylindrical shape and medium height plants (90 cm) mostly red purple leaf base. The color of leaves and petioles was observed to be green in most of the varieties except in a few where the petiole was dark green or with a red-purple touch and venation. In TAN/IND/13, adaxial and abaxial surfaces of the leaf were dark green and purple, respectively. TAN/PHL/02 was the best among the Philippine varieties due to its dark purple leaves and red-purple petiole, used for culinary and soup making. PNG-06 was the tallest among two other Papua New Guinean varieties and comparable to Samoan varieties. No serious insect pest and diseases observed in any of the 23 varieties tested at the As Perdido farm. Minor damage (1-2%) on crops by taro hornworms, aphids, ants, Chinese rose beetle, etc. was observed. Among the diseases, leaf spot (*Phyllostica colocasiophilla*) was observed in the varieties BL/SM/12, TAN/PHL/05, BL/PNG/08 and BL/HW/26. Soft rot of tubers (*Pythium* spp.) observed in the corms of most of the varieties except Japanese, when harvesting was delayed beyond 7 months.

Tissue culture

Tissue culture (TC) plants of selected varieties from the 1st trial were raised in NMC-CREES laboratory and sent to the islands of Rota and Tinian for preliminary trials. Shoot tips collected from the tissue culture plants grown at the As Perdido farm transferred into the MS medium begun to develop after 2-3 weeks' culture. One or two buds regenerated from one shoot tip. After 4 months of culture, about 83% of isolated tips regenerated shoots and roots on the same medium (**Fig. 3**). Protocorm-like bodies were produced on MS medium with IAA (1.0 mg/l) and Kinetin (1.0 mg/l) without shoot formation The protocol of regeneration from shoo-tips of taro was adopted from the Matsumoto's work conducted on dasheen taro (*Colocasia antiquorum* Schott). Advantages of TC is well-recognized such as plantlets are: i) uniform planting material, ii) clean and free of diseases, iii) planting material can be generated in large volumes for any planting season and farm size and iv) high yielding and quality corms (Morishita 1988).

Taste testing

Results of survey on taste testing of boiled corms, texture, acceptability and taste of all the varieties is shown in **Table 4**. The Japanese varieties rated excellent in taste, quality, smaller corm size, easy cooking, peeling and soft texture. All three Japanese varieties were the most favorable of all 23 accessions in terms of taste and texture. Small size, handling, shorter time in cooking and excellent taste are the

Table 4 Results of taste testing of boiled taro varieties conducted in Saipan (Jan. 2008 and 2009) and in Rota (Aug. 2008).

Crop	RGC Acc No.	Color	Taste	Texture	Acceptability
Taro (*Colocasia esculenta* var. *antiquorum*)	CA/JP-01	White-cream	Best	Soft, moist	Favorable
	CA/JP-06	White cream/purple	Best	Soft, moist	Favorable
	CA/JP-08	White cream	Best	Moderate hard, moist	Favorable
Taro (*Colocasia esculenta* var. *esculenta*)	BL/SM/04	Pale yellow	Fair (itchy)	Soft, moist	Moderately acceptable
	BL/SM/12	Light purple	Good	Hard, moist	Moderately favorable
	BL/SM/22	White-cream	Good	Dry, moist	Acceptable
	BL/SM/31	Light purple	Good	Hard, dry	Acceptable
	BL/SM/47	Light purple	Good	Dry, itchy	Acceptable
	TAN/MAL/07	White cream	Good (itchy)	Hard, moist	Acceptable
	TAN/MAL/08	Pale yellow	Fair (itchy)	Hard, moist	Acceptable
	TAN/MAL/12	Yellow	Good	Dry	Moderately favorable
	TAN/IND13	Pale yellow	Good	Hard, moist	Moderately favorable
	TAN/IND/19	White-cream	Good	Hard, dry	Acceptable
	TAN/IND/ 20	Yellow	Good	Soft, moist	Acceptable
	TAN/IND/23	Yellow	Good	Moderate hard, moist	Acceptable
	TAN/PHL/02	Dark purple	Good	Soft, dry	Acceptable
	TAN/PHL/05	Pale yellow	Good	Hard, dry	Acceptable
	TAN/PHL/15	Pale yellow	Good	Hard, dry	Moderately acceptable
	BL/PNG/03	Light purple	Good	Soft, dry	Moderately acceptable
	BL/PNG/06	Purple	Good (flavor)	Soft, moist	Moderately favorable
	BL/PNG/08	White cream/purple	Good	Soft, moist	Acceptable
	BL/HW/12	Dark purple	Good	Moderately hard, fibrous	Acceptable
	BL/HW/26	Light purple	Good	Dry, prickly sensation	Acceptable

Fig. 4 Cooked (boiled) taro var. TAN/IND/20.

Fig. 5 'Taste testing' of 23 var. of cooked taro at the As Perdido farm, Saipan.

reasons of most acceptability of the three varieties (CA/JP-01, 02 and 08) by consumers. All three Papua New Guinean (BL/PNG/03, 06 and 08) and Indonesian variety TAN/IND/13 was rated higher among other Indonesian varieties (TAN/IND/19, 20 and 23) in terms of taste and soft and moist texture (**Fig. 4**). Hawaiian varieties tasted good, however, dry and prickly sensation in mouth reported in the survey by some respondents (**Fig. 5**). Malaysian, Philippines and a couple of Samoan varieties were moderately acceptable due to itchy taste and hardness, dry texture. Out of three Malaysian cultivars, TAN/MAL-12 rated excellent in eating quality by taste testers (**Fig. 6**); however, TAN/MAL-7 and TAN/MAL-8 rated as fair or poor eating quality. Philippine taro (TAN/PHL-2, 5 and 15) grew very well in the first few months in the field but was rated poor and low yielding (**Fig. 7**). Samoan varieties were preferred due to big corms and more edible part, however, rated moderate due to itchiness and strong aroid taste. This quality was overcome by cooking style in some south Pacific islands and has become a favored variety.

Fig. 6 Cooked (boiled) taro var. TAN/MAL/12.

Fig. 7 Cooked (boiled) taro var. TAN/PHL/02.

DISCUSSION

All 23 varieties of taro imported survived in local soil and climatic conditions on the three islands of the CNMI, Saipan, Rota and Tinian. Although, soil type is classified as clay in the experimental sites, however, the cropping history, cultural practices and other environmental factors were different, including pest and diseases pressures and other variables that influence plant performance. No consumer preference was observed for the color of cooked varieties; however, a purple skin and cream flesh were favorable. Japanese varieties (CA/JP/01, 06 and 08) rated excellent among all the 23 varieties evaluated in the CNMI because of their taste and quality. Short plants (low-growing) was an added advantage in storms and frequent typhoons. Because of the frequency of typhoons, taller varieties are heavily damaged by storms in the CNMI.

The TC program in the CNMI is new and in an early stage; however, initial results obtained hold great promise for the farming community and in significant gains in the adaptability of many varieties by selecting and propagating

superior varieties using *in vitro* systems. We plan to continue taro variety trials to further evaluate selected lines and propagate through TC for distribution to the farming community.

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