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SEED RESCUE CULTURE: A TECHNIQUE FOR THE REGENERATION OF TARO (*COLOCASIA ESCULENTA*)

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ABSTRACT

In Papua New Guinea, twelve local taro cultivars were used to establish an initial breeding population from naturally flowering plants. Flowering rate varied significantly among cultivars, ranging from 0–5 flower heads per plant. Selected plants were transplanted and placed randomly in a separate plot to allow cross-pollination and production of fertile seeds to generate genetic variability and new recombinations in taro. At maturity, each flower head produced 400–500 seeds. The seeds did not grow in soil. However, embryos were rescued and grown aseptically via *in vitro* seed rescue culture (SRC) technique. Regenerated plantlets were free of contaminants and expressed a wide range of morphological and physiological variability. The SRC technique may be used for safer and convenient international exchange of taro germplasm and to widen the genetic diversity in taro breeding. The process encourages the initiation of a breeding programme involving hybridization of local and exotic cultivars to generate improved cultivars of taro.

Key Words: *Colocasia esculenta*, regeneration, seed culture, taro

RÉSUMÉ

En Nouvelle Guinée, douze cultivars locaux de taro ont été retenus pour constituer une population initiale de sélection à partir de plantes fleurissant naturellement. Le taux de floraison varie de manière significative parmi les variétés, de 0 à 5 fleurs par plante. Les plantes choisies ont été transplantées et disposées au hasard dans une parcelle pour permettre une pollinisation croisée et la production de graines fertiles afin de générer une variabilité génétique et de nouvelles recombinaisons de taro. A maturité, chaque fleur produit 400 à 500 graines. Celles-ci n'ont pas poussé dans le sol. Cependant les embryons ont été récuperés et faites pousser aseptiquement grâce à une technique de culture *in vitro*. Les plantules regénérées étaient libres de toute contamination et ont exprimé une gamme étendue de variabilité morphologique et physiologique. La technique de culture *in vitro* peut être utilisée pour un échange international approprié et plus sécurisant de germplasme de taro et pour élargir la diversité génétique dans la sélection du taro. Le processus encourage l'initiation d'un programme de sélection impliquant le croisement de cultivars locaux et exotiques pour générer des variétés améliorées de taro.

Mots Cés: *Colocasia esculenta*, regénération, culture de grains, taro

INTRODUCTION

Taro, *Colocasia esculenta* (L.) Schott, is a major staple food of the people of Papua New Guinea (PNG) and is a source of cash income for most small farm holders. Production of taro is declining at different altitudes of PNG. This decline is attributed to a number of factors among which

cultivar susceptibility to viral and fungal diseases is the most significant. Despite efforts to control these diseases, as much as 50% yield loss occurs. There are no published reports of taro breeding and selection in PNG.

The propagation of taro is exclusively by vegetative means, which restricts the development of genetic variability. Moreover, selection against disliked, low yielding, and flowering cultivars, and loss of germplasm collections due to diseases, probably have narrowed the taro genetic base in PNG.

Taro breeding requires sexual propagation through cross pollination. Until recently, there have been no published reports of taro breeding and selection (Strauss *et al.*, 1980). However, the recent development in seed germination and establishment of seedlings (Jackson *et al.*, 1977; Strauss *et al.*, 1980; and Wilson, 1979) has opened up possibilities. Breeding programmes have been initiated in the Solomons, Fiji, Western Samoa and Hawaii to provide farmers with improved cultivars of taro (Lebot and Aradhya, 1992).

Sexual reproduction of taro would permit recombination and generate genetic variability. Despite the natural occurrence of taro seeds from cross-pollination, sexual reproduction received little or no attention previously (Kikuta *et al.*, 1938; Porterfield, 1944). This is because of an apparent inability of seeds to germinate and/or seedlings to survive in the field (Strauss *et al.*, 1979).

Natural flowering in taro is limited to some PNG cultivars. Others fail to flower probably as a result of selection against flowering plants because of the belief that flowering reduces the harvested yield of taro (Shaw, 1975). Induction of flowering in non-flowering taro is possible using gibberellic acid (Alamu and McDavid, 1978; Wilson, 1979).

We are reporting an attempt towards utilization of taro seeds and regeneration of seedlings with new genetic recombinations and wide range of variability. This should allow hybridization for taro improvement and to ease maintenance of taro gene banks.

A schematic overall view of our taro research task and preservation of its genetic base in PNG is shown in Fig.1. The scheme should be useful to other fellow breeders who are interested in taro improvement. A taro gene bank (GB) may include

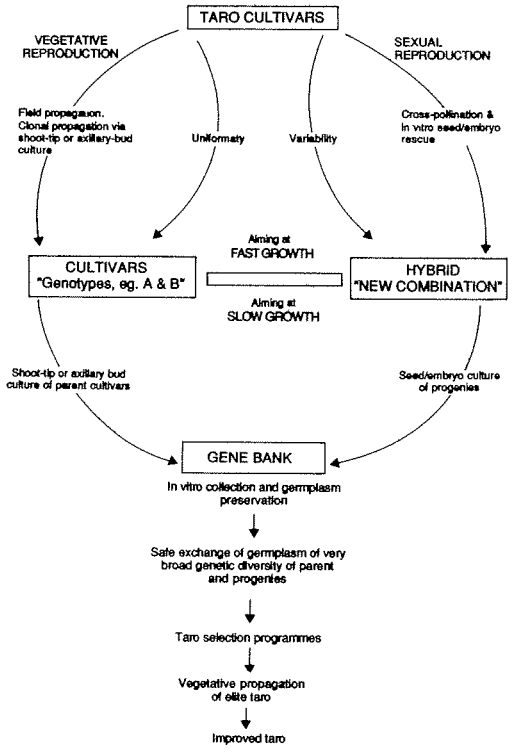

Figure 1. A model scheme for taro germplasm conservation and enhancement.

clones of local and exotic taro cultivars, and lines of variable, sexually reproduced taro. In PNG, taro is genetically diverse providing a wealth of variability for taro breeders around the world. However, the loss of germplasm collections in PNG (Bourke, 1979), selection against flowering taro, and initiation of selection in taro are likely to narrow the genetic base of this precious crop. Therefore, the scheme came about to meet a growing need for broadening the genetic base of taro and developing an alternative preservation method of its diverse germplasm in a gene bank. Clearly, regeneration of taro from seeds should be beneficial in this respect. A total of 121 flowering plants were selected, transplanted and maintained in one separate plot at the farm of PNG University of Technology in Lae.

MATERIALS AND METHODS

Naturally flowering plants of taro were selected from a collection of 12 cultivars grown at the farm of the University of Technology, Lae, Papua New Guinea. Eleven cultivars are coded with PNG numbers 5, 8, 13, 23, 29, 30, 34, 42, 77, 87, 89. The twelfth is a local cultivar called "Nanko". Each cultivar was represented by 160 plants. The plants with young flowering heads were transplanted in a separate plot at a spacing of 60x60 cm. All plants demonstrated symptoms of taro leaf blight, Alomae, and Bobone diseases. These flowering plants were used as a source of seeds in this study.

Mature taro fruits, after the spadix had dried or fallen off, were used as a source of seeds. Seeds were extracted either from fresh fruits or fruits dried at 34°C in a dry-air oven. Seeds from fresh fruits were squeezed off using a needle and scalpel. Seeds from dried flower heads were removed by crushing dried flower heads and passing seeds through a tea strainer into a beaker. Seeds were stored and tested for retention of their viability through germination trials. Sterilization of soil, filter paper, H_20 and Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was carried out by autoclaving at 15 psi (120°C) for 20 minutes. Seeds were surface sterilized by immersion in 1.5% Na-hypochlorite for 10 minutes. Germination was evaluated by counting the number of germinated seeds weekly until no further increase in seedling number was found.

TABLE 1. Flowering characteristics of naturally flowering taro cultivars at the farm of Papua New Guinea University of technology

Cultivar	No. of flowers/ plant	No. of Flowering plants (%)	Total no. of flowers
1. PNG 5	1	5	5
	2	16	32
	3	14	42
	4	11	44
	5	3	15
	Total	49 (30.6%)	4138
2. PNG 30	1	6	6
	2	2	4
	3	0	0
	4	0	0
	5	0	0
	Total	8 (5%)	10
3. PNG 34	1	15	5
	2	11	22
	3	8	24
	4	1	4
	5	0	0
	Total	25 (15.6%)	55
4. PNG 87	1	7	7
	2	7	14
	3	11	33
	4	0	0
	5	1	5
	Total	25 (15.6%)	54
5. Nanko	1	3	3
	2	8	16
	3	2	6
	4	0	0
	5	1	5
	Total	14 (8.7%)	30
	Grand total	121	287

Seedlings measuring 10–12 cm (with roots) were transferred to autoclaved soil in 10-cm pots, covered with a clear plastic bag, and placed in a greenhouse. When total plantlet length exceeded 30 cm, they were transferred to non-sterilized soil in large pots and kept in the greenhouse for observation.

RESULTS AND DISCUSSION

Only five cultivars produced flowers in 6–8 weeks after planting with a range of 1 to 5 inflorescences per plant. Because taro is believed to be incapable of self-pollination (Wilder, 1923; Show, 1975; Strauss *et al.*, 1986), close spacing of flowering

TABLE 2. Parameters of naturally occurring flowers of five cultivars of taro growing at the farm of Papua New Guinea University of Technology.

Cultivar entry no.	Size of female part (mm)	Number of ovaries				No. of seeds/ ovary (range)	Total no. of seeds/ flower head
		Total	Infertile	Fertile without seeds	Fertile with seeds		
1. PNG, 5	14 x 50	210 ± 6	9 ± 2	23 ± 2	178 ± 5	0–13	420 ± 20
2. PNG, 50	21 x 53	197 ± 5	14 ± 2	16 ± 2	167 ± 4	1–13	430 ± 31
3. PNG, 34	16 x 48	213 ± 4	13 ± 3	32 ± 2	168 ± 5	0–18	470 ± 18
4. PNG, 87	18 x 39	191 ± 9	16 ± 3	25 ± 2	150 ± 12	1–14	397 ± 9
5. Nanko	19 x 53	173 ± 5	20 ± 2	50 ± 2	104 ± 4	3–14	450 ± 9

[a]Three randomly selected flower heads were used from each cultivar; the data shows the mean values and ± indicate the standard errors.

TABLE 3. Factors affecting germination of taro seeds extracted from a flower of "Nanko" cultivar

Source of flower head	Germination media	Treatment		Germination rate (%)	Fungal contamination
		seeds	Media		
1. Flowering plant in population	Topsoil	SS	A	0	No
	H_2O	SS	NA	0	Yes
		NS	A	0	No
		NS	NA	0	Yes
	H_2O, filter paper	SS	A	86	No
		SS	NA	58	Yes
		NS	A	76	Yes
		NS	NA	0	Yes
	Liquid MS filter paper	SS	A	93	No
		SS	NA	0	Yes
		NS	A	0	Yes
		NS	NA	0	Yes
	MS-agar	SS	A	98	No
2. Flowering plant in isolation	MS-agar	SS	A	0	No

plants was thought to be essential to allow for cross-pollination and viable seed setting. Table 1 presents the flowering characteristics of the 121 selected plants of taro. Frequency of number of flowers per plant increased with the increase in flowering rate (number of flowering plants/total number of plants). The cultivar PNG 5 had significantly ($P \leq 0.05$) the highest flowering rate and accordingly, the highest frequency of flowers. On the other hand, the cultivars PNG 30 and "Nanko" had significantly ($P \leq 0.05$) lower flowering rate and frequency of flowers, while the cultivars PNG 34 and 87 were intermediate. This association may be attributed to certain factors controlling flowering in taro. The extraction of taro seeds from ovaries of flower heads after drying at 34°C in a dry air oven was much easier and quicker than from freshly-cut flower heads. Extracted seeds were maintained in petri-dishes under the same drying conditions. Using a stereo-microscope, it was seen at 40X magnification that taro seeds were polymorphic in size and shape within each flower head. This variability may reflect heterozygosity. The established method of extraction is simple and could be mechanised for large scale seed extraction.

Table 2 shows that flower heads varied slightly in size. The total number of seeds/flower ranged from 380 to 511. Some ovaries had ovules but no seeds. Apparently, these ovaries are fertile but may not have been pollinated (Show, 1975).

Germination rate of seeds from freshly-cut flower heads varied according to source of seed, growth media, and sterilization condition of both

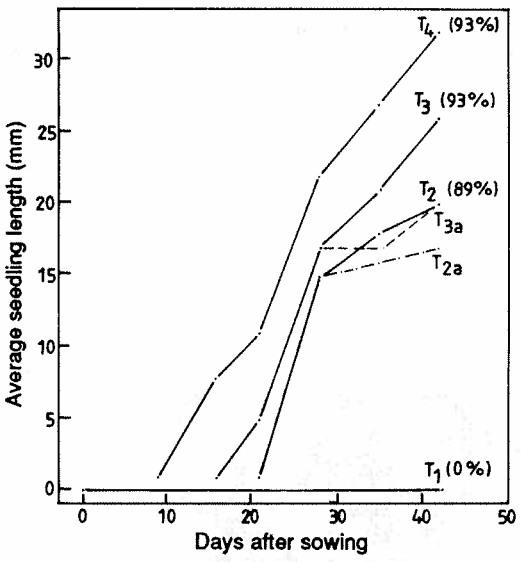

Figure 2. The effect of media on germination and seedling growth of "Nanko" cultivar of taro. Data are means of 100 seeds per treatment [T].

- T_1: Seeds sown in top soil in H_20 for 42 days
- T_2: Seeds sown on H_20 wetted filter paper for 42 days
- T_{2a}: Seeds sown on H_20 wetted filter paper for 28 days, then transferred from topsoil to H_20 for another 14 days
- T_3: Seeds sown on wetted MS-wetted filter paper for 42 days
- T_{3a}: Seeds sown on MS wetted filter paper for 28 days then transferred for topsoil/H_20 for another 14 days
- T_4: Seeds sown on MS-agar medium

seeds and the growth media (Table 3). On several occasions when a taro plant of "Nanko" cv. with a single flower was found in isolation from other flowering plants, none of its seeds germinated. However, germination rate was 98% when seeds were extracted from flowering taro plants of "Nanko" cv. grown in the flowering breeding collection. This suggests that taro, to a large degree, is cross-pollinated or that pollen grains have to come from another plant or at least from another flower.

Sterilization of seeds and germination media was essential to limit fungal contamination that significantly affected the germination rate. Fungal contamination was heaviest when unsterilized nutrient media were used for germination. Surface sterilized seeds did not germinate in autoclaved damp topsoil or in the field. However, the germination rate was 86% on autoclaved damp filter paper and 93% on filter paper wetted with autoclaved liquid MS medium. The highest germination rate of taro seeds (98%) occurred when MS-agar medium was used following normal procedure of tissue culture. This medium was found to be the most supportive for germination.

On MS-agar medium, the seeds germinated 9 days after sowing but took 21 days to germinate on damp filter paper and 16 days on filter paper wetted with liquid MS medium (Fig. 2). Seedlings with an average length of 16 mm at 28 days of age were transferred to autoclaved topsoil and grown under high relative humidity conditions. After 14 days on soil (42 days old), the length of the

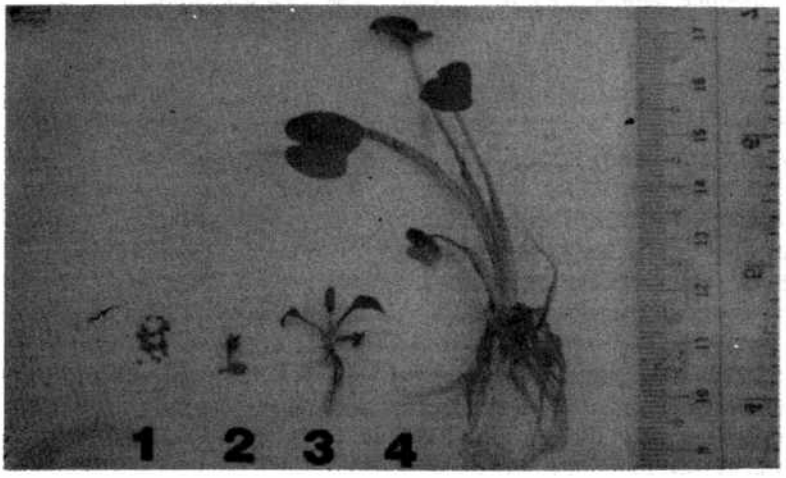

Figure 3. Stages of growth and development of taro seedlings grown on MS-agar medium for seed rescue. Stage (1): seeds; (2) two-week-old germinated seed with a circular bilobed cotyledon leaf and two roots emerging out of the seed testa; (3) 4-week-old seedlings with three successive leaves and few roots; and (4) 8-week-old seedling immediately out of *in vitro* conditions, ready to be transferred to soil.

TABLE 4. The effect of storage conditions on the viability of taro seed

Storage conditions				% germination following storage period (days)				
Temp. (°C)	Humid.	Storage container	Place of storage	0	10	30	60	90
4 ± 1	Humid	covered Petri-dish	Fridge	–	92	84	76	67
22 ± 2	Humid	Petri-dish covered with para film	Bench	–	72	58	41	–
34	Dry	Uncovered Petri-dish	Oven	–	97	96	96	98
Control	(Freshly extracted seed)			96	–	–	–	–

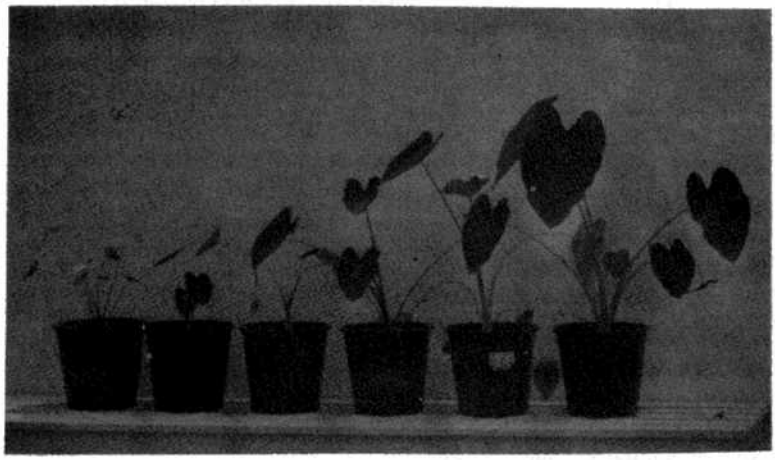

Figure 4. Five-month old taro plants regenerated from seeds of one flower head of "Nanko" *cv.* under uniform environment. The plants show a range of morphological variability.

seedling had increased by only 2–3 mm, whereas after the same period on MS-agar medium the length of the seedling increased by 10–12 mm. When seeds were cultured on MS-agar medium supplemented with 0.5 mgl^{-1} NAA, the length of the seedling reached 50 mm at the age of 42 days.

This seed rescue culture was the fastest and easiest way of germinating taro seeds and regenerating seedlings and it supports their growth until they reach approximately 10 cm. Figure 3 shows the stages of growth and development of seedling from taro seeds. Seedlings at the 10 cm stage were transferred to soil and maintained in a greenhouse.

Freshly extracted seeds were used as a control and gave a germination rate of 96%. Storage of seeds at 34°C did not affect their viability. The germination rates tested four times over a period of 90 days were equivalent to that of the control (Table 4). Storage of seeds for 90 days in a fridge (4°C) caused a gradual decline in the germination rate with a loss of 30% of the seed viability. Storage at room temperature (22°C) reduced germination sharply.

In addition to the polymorphic characteristics of seeds, the morphological analysis of seedlings also indicated a wide range of variability. Figure 4 shows a sample of five-month-old taro plants regenerated from seeds of one flower head of "Nanko" cv. under uniform environment. Variability in growth vigour, number and colour of petioles, leaf size and shape, size and nature of root growth, and response to pests and diseases were observed and are presently under assessment. The occurrence of this variability under uniform environment suggests a high degree of heterozygosity.

CONCLUSION

Germination of PNG taro seed after long term storage and the occurrence of a wide range of

variability in seedlings make taro improvement possible through selection and hybridization. The establishment of methods for taro seed storage and recovery of seedlings through *in vitro* culture should provide an excellent means for preserving the PNG taro gene bank. Moreover, seeds and *in vitro* growing seedlings should be of great quarantine value, particularly if taro viruses are not seed borne.

Cross-pollination in taro is likely to be common. This is supported by failure of seeds from a flower in isolation to germinate and the occurrence of a wide range of variability in seeds and in regenerated seedlings. However, pollination that may occur within a clone and even within the same monoecious flower of taro is considered selfing (Lebot and Aradhya, 1992). The wide range of variability indicates that self-pollination in taro is very rare (Wilder, 1923).

These results should be beneficial for a successful taro improvement programme, international collaboration, and *in vitro* preservation of recombinants of the existing cultivars in Papua New Guinea and elsewhere.

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