Seed germination and seedling development in Taro (*Colocasia esculenta*)

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ABSTRACT

Two taro (Colocasia esculenta (L.) Schott var. esculenta) cultivars from Fiji and Papua New Guinea were grown at the University of the South Pacific, Laucala Campus, Fiji to produce seeds for seed storage experiments. Gibberellic acid at a 500ppm concentration was used to induce flowering. Very few flowering shoots (inflorescence) were observed in the Fiji cultivar and all pollinations were unsuccessful. However the PNG cultivar flowered well and was used to obtain seed after hand pollination. Hand pollination was carried out to ensure seed setting in developing fruits in the inflorescence. Seeds were extracted in the laboratory after harvesting mature inflorescences. Experiments were conducted on seed moisture content, desiccation, germination, seedling development and seed storage behaviour of taro (Colocasia esculenta) seeds. Seed moisture content was determined using oven methods and air-drying. Results demonstrated that taro seeds have a moisture content of 12-13% after air-drying for three to four weeks. Seeds were dried to desired moisture contents in a desiccator over silica gel. After drying to 5% moisture content seed viability was tested by germinating seeds on moist filter paper at room temperature with 65% relative humidity and seven to eight hours day length. Preliminary seed germination tests demonstrated up to 83% germination for seeds with 13% moisture content. Germination occurred within five to seven days. Maximum germination was achieved within 21 days. The highest germination (80%) was achieved with seeds with 12% moisture content. Results indicated there was no relationship between moisture content and seed germination. Normal seedling development and growth was recorded after germination.

Ke words: *Moisture content, Dry weight, Gibberellic acid, Dasheen cultivars, Colocasia esculenta var esculenta*

1 INTRODUCTION

Taro belongs to the genus *Colocasia*, of the monocotyledonous family Araceae. The cultivated taro species is classified as *Colocasia esculenta*, but the species is considered to be polymorphic (Purseglove, 1972). There are at least two botanical varieties: (i) *Colocasia esculenta (L.) Schott* var. *esculenta, and* (ii). *Colocasia esculenta (L.) Schott* var. *antiquorum.*

Colocasia esculenta var.*esculenta* has a large cylindrical central corm and a few cormels (small side corms). Agronomically it is referred to as the dasheen type. *C. esculenta* var. *antiquorum* has a small globular central corm with several relatively large cormels arising from the main corm. This variety of taro is called (agronomically) the eddoe type. (Lebot and Aradhya, 1991; Purseglove, 1972). Most of the taro grown in the Asia-Pacific region is of the dasheen type.

Although taro is said to have originated in North Eastern India and Asia (Ivancic, 1992; Hanson and Imamuddin, 1983; Kuruvilla and Singh, 1980), recent phylogenetic studies have provided evidence that most cultivars were domesticated from wild sources existing in the New Guinea and Melanesian areas (Lebot, 1992). Taro (*Colocasia esculenta*) is an important food crop in many Pacific Island countries and is grown for its underground stem (rhizome called corm) as a root crop (Ivancic, 1992). The corm is an excellent source of carbohydrate but low in fat and protein, and the taro leaves provide carotene and potassium. (Bradbury and Holloway, 1988; Hanson and Imamuddin, 1983; Lambert, 1982; Griffin, 1982). It has played an important role in the peoples' customs and traditions in the Pacific, and has attained considerable economic importance as a fresh crop in many of the island countries such as Samoa, Fiji, Tonga and others (Hanson and Imamuddin, 1983).

Because of several diseases and pests, taro production is decreasing in many countries and is being replaced by sweet potatoes and cassava (Ivancic, 1992). In some countries, competing demands placed on labour to produce crops both for food and cash, have seen a trend towards the replacement of traditional cultivars by a smaller number selected or bred for high yield in monoculture (Guarino and Jackson, 1986). The loss of this traditional diversity may have serious repercussions. It may mean that in the face of serious pest outbreaks, or a need for other traits, such as nutritional quality, ecological adaptation including climatic changes, food processing potential, pharmaceutical products, or some as yet undefined need, cultivars will not be available to evaluate.

It was in response to this threat to taro germplasm, that AusAID funded the Taro Genetic Resources: Conservation and Utilization project, (TaroGen). The International Plant Genetic Resources Institute (IPGRI) provided technical support in developing and implementing this project. TaroGen worked with several countries in the Pacific, collecting taro and then rationalizing the collections. A core collection, representative of the genetic diversity in the entire collection, has been conserved *in vitro* at the Secretariat of the Pacific Community (SPC) Regional Germplasm Centre (RGC). TaroGen also considered other conservation methodologies in the development of a complementary conservation strategy (CCS) for taro. Seed storage was considered as a methodology useful for the conservation of taro genes, though not the specific gene combinations, which characterize different landraces. Therefore knowledge of seed storage behaviour is important. Movement of seeds for breeding programmes is also important, as this encourages utilization of germplasm.

Taro is mainly propagated using small suckers (Ivancic, 1992; Kuruvilla and Singh, 1980; Strauss *et al.* 1979; Shaw, 1975). Most taro cultivars do not flower, however it is now well documented that gibberellic acid promotes flowering in taro (Ivancic, 1992; Wilson, 1990; Hanson and Imamuddin, 1983) and techniques for pollinating and growing seedling population have

beenused in breeding programmes (Singh *et al.* 2001; Ivancic, 1992; Wilson, 1990; Hanson and, Imamuddin, 1983; Shaw, 1975).

Although reports indicate that taro plants can produce viable seeds under natural conditions, the number of seeds per fruit varies from very few to thousands (Ivancic, 1992; Wilson, 1990; Shaw, 1975; Strauss, 1979). Similarly some experimental evidence suggests that under appropriate conditions taro seeds will germinate fairly easily (Wilson 1990; Strauss *et al.* 1979; Shaw 1975), but there are other reports where seed germination has failed. Strauss *et al.* (1978) reported that taro seeds stored in airtight container (at normal room temperature) failed to germinate after a 30-day storage period and that seeds kept for various periods in a refrigerator or freezer did not germinate A preliminary study, carried out in PNG indicated that germination of taro seeds is affected by genotype, environmental factors, harvesting conditions, storage conditions and germination protocols. (Singh *et al.* 2001). Germination rates of 75-80% were obtained with seeds less than three months old, but only 40-50% was obtained when the seeds were over 12 months old. This all points to the need to identify best practices for handling seeds on harvesting, and to optimise germination methods. In addition, the orthodox behaviour of seeds should be confirmed using the protocol described by Hong and Ellis (1996). If conditions can be optimised, seed storage will offer a convenient method for long-term conservation of taro genes. In addition, it will offer an alternative avenue for the international movement of plant germplasm by minimising the risk of transmitting pathogens. This will be extremely useful for taro improvement programmes.

2 MATERIALS AND METHODS

Two taro cultivars (*Colocasia esculenta* var *esculenta*) from Fiji and Papua New Guinea were planted in August 2003 at the University of the South Pacific, Laucala Campus, Fiji. Normal agronomic practices including fertilizer application, weeding and other operations were carried out. During dry spells plants were watered twice a week.

2.1 FLOWER INDUCTION

When plants were three months old, gibberellic acid (GA) at 500ppm concentration was sprayed on the lower and upper surface of leaves of plants at the fifth leaf stage. Flowering commenced within one to two months after GA spraying and continued for three to four months. However the Fiji cultivar produced very few inflorescences. Pollen production was also very poor in this cultivar. Hand pollination was not successful and no seed set was observed, and therefore all seeds used in this study were collected from the PNG cultivar.

2.2 HAND POLLINATION

The male part of the flower was cut and removed. The spathe (boot leaf around female inflorescence) was carefully cut and removed to expose female flowers at the 'crack' stage (Wilson, 1990). Pollen from male flowers was dusted onto the stigma by means of a soft brush. After pollination, the female inflorescences were covered with a pollinating bag. Pollinating bags were removed after five days. Inflorescences were checked daily for ripening fruits containing the developing seeds. Ripening inflorescences were soft and succulent and the berries in the inflorescence turned green to pale yellow and became soft when touched.

2.3 SEED EXTRACTION AND SEED DRYING

Mature inflorescences were removed from plants, and placed in a very fine tea strainer. Berries were crushed, by rubbing against the surface of the strainer. The fruit pulp and lightweight seeds floating on the top were discarded. Heavy seeds were washed further with water to remove extra pulp. Finally, water was drained and the seeds were placed on filter paper in a petri dish and allowed to air-dry. After air-drying for three to four weeks damaged seeds and other cellular debris was removed. Healthy seeds were counted and packed in plastic bags.

2.4 MOISTURE CONTENT ANALYSIS

The initial weight of 400 seeds was recorded. The seeds were left in the oven at 105^0C for five hours. After drying, seeds were reweighed. The process of heating, cooling and reweighing was repeated until a constant weight was reached. The % Moisture Content (MC) of the seeds was calculated as follows:

$$\%MC = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

2.5 DESICCATION TRIAL

Seeds were desiccated to Desired Moisture Contents (%DMC) of 12%, 9%, 6% and 3% using silica gel in a desiccator. The final weight of seeds at the DMC was calculated using the formula:

$$\text{Weight of seeds (g) at \%DMC} = \text{Initial seed weight (g)} \times \frac{(100 - \text{Initial \%MC})}{(100 - \text{\%DMC})}$$

When the %DMC was obtained, samples of 50 seeds were taken and tested for viability by germinating.

2.6 GERMINATION TRIALS

50 seeds were humidified at 95% RH for 24 hours and then soaked on moist filter paper for another 24 hours. The seeds were surface sterilized by rinsing with 10% household bleach Whiteking™ (42 g sodium hypochlorite/ litre) for two minutes. After rinsing thoroughly with distilled water, the seeds were spread on moist filter paper in a petri dish and left at room temperature (27-28°C), RH 65% and seven to eight hours day length for germination.

2.7 CALCULATION OF GERMINATED AND NON-GERMINATED SEEDS

The total number of germinated seeds was counted and the germination percentage was calculated as follows:

$$\% \text{ Germination} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds Sown}} \times 100$$

All non-germinated seeds were tested for viability by tetrazolium staining. Embryos were removed and placed in 10 ml phosphate buffer (pH=7.0) for 10 minutes before transferring 10 ml of 0.1% tetrazolium. Embryos were then placed in the dark for 30 minutes.

3 RESULTS AND DISCUSSION

The number of seeds obtained from mature inflorescences of the PNG cultivar varied from one to ten per berry and from 15-2300 per inflorescence. Wilson (1990), Shaw (1975) and Wang (1983) have reported similar results and inferred that this mostly depended on the size of the berries and inflorescence.

A total of 12,946 seeds were collected from the 37 inflorescences (excluding the light and damaged seeds). After one month of air-drying the average weight of the seeds was 0.16 mg with the moisture content of 12 – 13%.

3.1 DESCRIPTION OF TARO SEED

Seeds collected in this study (Figure 1) have the features as described by Shaw (1975) and Wang (1983), and a mean weight of 0.16mg.

Figure 1. Dried mature taro seeds (Magnification X 720)

3.2 MOISTURE ANALYSES, DESICCATION AND GERMINATION

Moisture analysis of air-dried seeds (Sample 1 seeds were dried for 3.5 weeks) moisture content was 12.77% (Batch 1) and (sample 2 seeds were dried for 2.5 weeks) moisture content 13.14% (Batch 2).

Table1. Preliminary germination trials for taro seeds. With MC of 12-13% (M = mixture of both B1 & B2 seeds. B1 = seeds dried for 3.5 weeks. B2 = seeds dried for 2.5 weeks).

Batch No	Total number of seeds	Germinated Seeds	Germination %	Fresh non-germinated Seeds %	Dead Seeds %	Empty Seeds %
M	42	35	83%	4.76%	4.76%	7.14%
B1	48	19	40%	41.67%	8.33%	10.42%
B2	43	34	79%	2.33%	6.98%	11.63%

3.3 PRELIMINARY GERMINATION TRIAL

The moisture contents of the seeds were 12.77 and 13.14% after air-drying for 3.5 and 2.5 weeks respectively. The seeds were germinated on moist filter paper. Seeds began to sprout after seven days. Most of the seeds germinated within 21 days. A combination of seeds from batch one and two showed up to 83% germination, with germination rates of 79% (batch two) and 40% (batch one) from the individual batches. Wilson (1990) and Wang (1983) obtained similar results on taro seed germination. From the results it would seem that the germination achieved is not related to the original moisture content.

The examination of the non-germinated seeds at the end of germination period showed that some seeds (5-8%) were infected with fungus. About 7-11% seeds were found almost empty and therefore non-viable, whereas 2-41% seeds were viable but did not germinate possibly due to dormancy. Seeds were treated with or rinsed with bleach to remove and kill fungal spores. Fungal attacks and damping off are major problems in germinating taro seeds (Wang, 1983). Wang (1983) suggested that these problems are overcome by surface sterilizing the seeds with a saturated calcium hypochlorite solution (10g/140ml H_2O) for 10 minutes or 1% sodium hypochlorite and / or sowing the seeds in a sterile

medium. The various stages of seed germination and seedling development of taro seeds are shown in Figure 2.

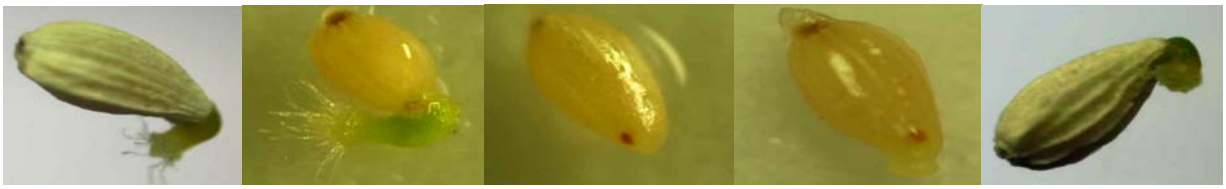
3.4 EFFECT OF SEED MOISTURE CONTENT ON GERMINATION

Taro seeds desiccated to 12%, 9%, 6% and 3% moisture content showed 80%, 72%, 68% and 78% germination respectively (Table 2). As previously stated there appears to be no relationship between seed moisture content and germination. This pattern of germination is indicative of orthodox behavior (i.e. seeds showing 70-80% germination with low to very low moisture contents (Hong and Ellis 1996).

Table 2. Germination of taro seeds desiccated to different moisture contents

Seed Sample	%DMC	Germination Test Results (Total = 100%)			
		Germinated Seeds % (50 Seeds)	Dead Seeds % (50 Seeds)	Empty Seeds % (50 Seeds)	Fresh Non-germinated Seeds % (50 Seeds)
PNG Variety	**Control 13.14%**	30	0	16	54
	12 %	80	0	4	16
	9 %	72	0	8	20
	6 %	68	2	10	20
	3 %	78	0	8	14

Fresh Seeds- *those, which imbibe and are firm and free from primary fungal infection.*
Dead Seeds - *those, which developed mould and when gently squeezed, exude liquid.*
Empty Seeds - *those, which do not have an embryo or are only partially developed.*



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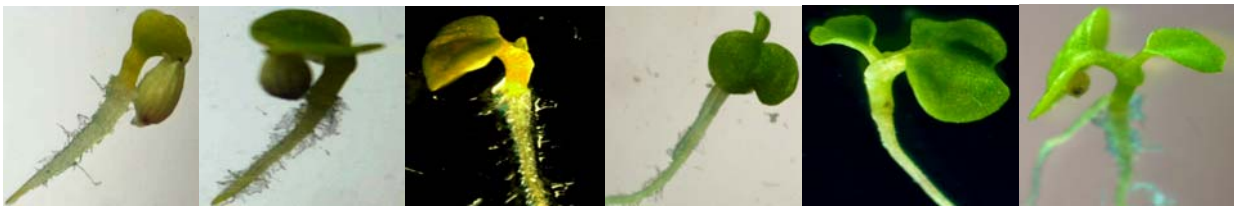

f g h I j k

Key. a -3 days, b - 5 days, c - 6 days, d - 7 days, e - 8 days, f - 9 days, g - 11 days, h - 20 days, i - 21 days, j - 28 days, k -43 days.

Staining of non-germinated seeds with tetrazolium showed that some seeds remained viable even after the end of the germination period but did not germinate (Table 1 and 2). As previously discussed this could be due to dormancy. However, Wilson (1990) reported that taro seeds germinate within seven to fourteen days after sowing with no apparent dormancy. Wang (1983) also stated that taro seeds do not lose viability easily, do not become dormant and can germinate after long period of time if stored properly. Under appropriate conditions germination begins seven days after sowing and majority of seeds germinate within 21 days. In the present study germination started on day five and most of the seeds had germinated within three weeks. Therefore taro seed dormancy is unlikely to be the reason why the viable seeds failed to germinate.

Wang (1983) and Strauss *et al.* (1979) observed that seeds weighing less than 0.2mg did not germinate.

However, in the present investigation taro seeds weighing 0.16mg, had germination rates to 83% (Table 1 and 2). This favourable response from seeds of relatively low weight could be due to seed vigour, varietal differences and/or the pre-germination treatment given to the seeds in this study.

4 CONCLUSION

Traditionally taro is vegetatively propagated plant species but some species flower and produce seeds naturally while others can be induced to produce seeds by spraying with gibberelic acid. Taro seeds should be dried preferably at room temperature to reduce moisture and to ensure viability is sustained for a long period of time. Seeds in the present investigation were air-dried, desiccated and tested for germination and seedling growth. Seed weight did not appear to affect seed germination and seedling growth. The maximum germination achieved was 83% and after germination seedling growth was normal. Reducing the moisture content of the seed to 3-12% did not affect germination. The taro seed viability and germination tests carried out in this study indicate that taro seeds are orthodox, however, this orthodox behaviour of taro seeds will be confirmed by storing the seeds hermetically at various temperatures (ambient laboratory temperature; 27^0C to -196^0C in liquid nitrogen for different durations (up to one year). Seeds, which tolerate desiccation to 3% moisture contents, do not necessarily show orthodox behaviour. Investigations of seed survival in different storage environments are required to determine seed storage behaviour

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