Improving taro (*Colocasia esculenta* var. *esculenta*) production using biotechnological approaches

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Abstract

Taro (*Colocasia esculenta* L. Schott) is an important crop worldwide but is of particular significance in many Pacific Island countries where it forms part of the staple diet and serves as an export commodity. Escalating pest and disease problems are jeopardizing taro production with serious implications to food security and trade. Biotechnological approaches to addressing pest and disease problems, such as transgenesis, are potentially viable options. However, despite biotechnological advancements in higher profile agronomic crops, such progress in relation to *Colocasia esculenta* var. *esculenta* has been slow. This paper reviews taro biology, highlights the cultural and economic significance of taro in Pacific Island countries and discusses the progress made towards the molecular breeding of this important crop to date.

Key words
Taro, *Colocasia esculenta*, biotechnology, transgenic

Introduction to taro – a monocotyledonous root crop

Taro is an important staple food crop grown throughout many Pacific Island countries, parts of Africa, Asia and the Caribbean for its fleshy corms and nutritious leaves. In addition to contributing to sustained food security in the domestic market, it also brings in export earnings (Revill *et al.*, 2005). The plant can fit well into tree crop and agro forestry systems and some types are particularly well adapted to unfavourable land and soil conditions such as poor drainage. As such taro is grown under intensive cultivation as a starch crop (Jianchu *et al.*, 2001).

Morphology, botany and genetics

Taro (*Colocasia esculenta*) is a herbaceous plant, which grows to a height of 1-2 m. The plant consists of a central corm lying just below the soil surface, with leaves growing from the apical bud at the top of the corm and roots growing from the lower portion. Cormels, daughter corms and runners grow laterally. The leaf is peltate; the root system is fibrous and lies mainly in the top one metre of soil. The corm is a nutrient storage organ and shares the following characteristics with food storage organs in carrot, sweet potato and manioc: abundance of periderm, food storage in large, thin-walled parenchymatous cells, poorly developed vascular bundles that are few in number,

presence of latex cells, mucilage cells and ergastic substances such as druses and raphides (Miyasaka, 1979).

Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be polymorphic (Purseglove, 1972). There are eight recognized variants within *Colocasia esculenta*, of which two are commonly cultivated (O'Sullivan *et al.*, 1996): i) *Colocasia esculenta (L.) Schott* var. *esculenta* which possesses a large cylindrical central corm and only few cormels (Figure 1A); agronomically it is referred to as the 'dasheen' type of taro and ii) *Colocasia esculenta (L.) Schott* var. *antiquorum* which has a small globular central corm with several relatively large cormels arising from the corm (Figure 1B); agronomically this variety is referred to as the 'eddoe' type of taro (Purseglove, 1972; Lebot and Aradhya, 1991). Most of the taro grown in Asia Pacific region is of the dasheen type. In places where taro is grown primarily for leaves, *C. esculenta* var. *antiquorum* is preferred (O'Sullivan *et al.*, 1996).

Chromosome numbers reported for taro from various regions include 2n = 22, 26, 28, 38 and 42 (Onwueme, 1978). The most commonly reported chromosome numbers are: diploids 2n = 28 and triploids 3n = 42 (Kuruvilla and Singh, 1981; Wang, 1983; Lebot and Aradhya, 1991; Lee, 1999). Furthermore, plants with 3n = 42 are referred to as *alowane* (male, large plant) and those of 2n = 28 are referred to as *alokine* (female, short plant) by Solomon Island farmers (Jackson *et al.*, 1977; Wang, 1983).

Figure 1: (A) *C. esculenta* var. *esculenta* (dasheen) has a large central corm and (B) *C. esculenta* var. *antiquorum* (eddoe) has a small central corm with multiple relatively large cormels.

Origin and distribution

Taro is thought to have originated in North Eastern India and Asia (Kuruvilla and Singh, 1981; Hanson and Imamuddin, 1983; Ivancic, 1992) and gradually spread worldwide by settlers. As such, it is now cultivated in more than 65 countries worldwide (USDA, 2001). Using isozyme analysis, Lebot and Aradhya (1991), reported the existence of two gene pools for cultivated taro; one in Asia and the other in Pacific. Studies with SSR markers (Simple Sequence Repeats) (Noyer *et al.*, 2003) and AFLP markers (Amplified Fragment Length Polymorphism) (Kreike *et al.*, 2004) have confirmed the existence of these two distinct gene pools. This indicates that taro was domesticated in Asia as well as in the Pacific; therefore, it can be considered as a native plant of the Pacific.

Nutrition

Taro corm is an excellent source of carbohydrate, the majority being starch of which 17-28% is amylase, and the remainder is amylopectin (Oke, 1990). The size of taro starch grain is one-tenth that of potato and its digestibility has been estimated to be 98.8%. Because of its ease of assimilation, it is suitable for persons with digestive problems. Taro is especially useful to people allergic to cereals and can be consumed by children who are sensitive to milk, and as such taro flour is used in infant food formulae and canned baby foods (Lee, 1999). Taro corm is low in fat and protein; however, the protein content of taro corm is slightly higher than that of yam, cassava or sweet potato. The protein is rich in some essential amino acids, but is low in isoleucine, tryptophan and methionine (Onwueme, 1978). Taro leaves contain higher levels of protein and are also excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibre (Onwueme, 1978; Lambert, 1982; Hanson and Imamuddin, 1983; Bradbury and Holloway, 1988; Opara, 2001). They also contain greater amounts of vitamin B-complex than whole milk (Lee, 1999) and are higher in protein than that of tannia (new cocoyam; *Xanthosoma sagittifolium*) and all other nutrients except oil. The fresh taro leaf lamina and petiole contain 80% and 94% moisture, respectively.

Economical importance and uses of taro

In Pacific Island countries such as Fiji and parts of Africa, taro is a staple food crop (Lebot and Aradhya, 1991; Opara, 2001). In Tonga, for example, tubers represent almost half the nations' calory intake of which about 40% is contributed by taro. Similarly, in Solomon Islands, about 10% of people's dietary calories come from taro and 30% from other tubers. Moreover, in Samoa, prior to a devastating spread of taro leaf blight (TLB), virtually all the populations' dietary intake from tubers (one-fifth of the overall diet) came from taro (CTA, 2003). Taro is one of the few major staple foods where both the leaf and underground parts are important in the human diet (Lee, 1999). As such, it has attained considerable economic importance as a fresh crop in many large islands in the region such as Samoa, Fiji and others (Hanson and Imamuddin, 1983). It is now becoming one of the major export commodities providing substantial foreign exchange to some of the Pacific Island countries.

Large quantities of taro are produced in Asia/Pacific region, with the corm being boiled, baked or fried and consumed with fish and coconut preparations. A favorite and peculiarly Pacific way to prepare taro is to roast it on hot stones in dug-out earth ovens. This is quite common when taro is used in feasts and ceremonies. Young taro leaves are used as a main vegetable throughout Melanesia and Polynesia where they are usually boiled or covered with coconut cream, wrapped in banana or breadfruit leaves and cooked on hot stones. The processed and storable form of taro is the taro chip and Poi. Taro chips are prepared by peeling the corm, washing, slicing into thin pieces and blanching; the pieces are then fried in oil, allowed to cool, then drained and packed. Poi is a sour paste made from boiled taro and its production and utilization is quite limited – mainly in the Hawaiian Islands.

Griffin (1982) has emphasized other important economic uses of taro. For example, the development of taro silage and its use as animal feed especially for swine, the potential of taro alcohol as a fuel for remote islands and the potential of taro starch as a raw material in cosmetic and plastic manufacture. Furthermore, taro flour and other products are used extensively for infant formulae in the United States and have formed an important constituent of proprietary canned baby foods (Lee, 1999).

Cultural importance of taro

Taro has evolved with the cultures of the people of the Asia and Pacific region; therefore, it has acquired considerable socio-cultural importance. It is considered a prestige crop and the crop of choice for royalty, gift-giving, traditional feasting and the fulfillment of social obligations. It features prominently in the folklore and oral traditions of many cultures in Oceania and South-east Asia. Samoa and Tonga each have prominent depictions of taro on their currencies (Onwueme, 1999). Moreover, in Hawaii, images of taro and taro farmers can be found throughout the islands, in murals, posters, original arts and other visuals, where its symbolic importance reflects its continuing role as a common food and common element in the agricultural landscape (Matthews, 1998). The socio-cultural attachment to taro has meant that taro itself has become a symbol of cultural identification, such that the people of Pacific Island origin continue to consume taro wherever they may live in the world. This is one of the means of maintaining links with their culture; consequently, this cultural attachment to taro has spawned a lucrative taro export market to ethnic Pacific Islanders living in Australia, New Zealand and western North America (Onwueme, 1999). Taro is also used as a traditional medicine with root extract used to treat rheumatism and acne, while leaf extract is used for blood clotting at wound sites, neutralizing snake poison and as a purgative medicine (Thinh, 1997).

Diseases and pests of taro

In many countries taro is being replaced by sweet potatoes and cassava largely due to pests and disease problems, which are becoming a limiting factor for taro production (Ivancic, 1992).

Viruses are one of the most important pathogens with some infections resulting in severe yield reductions and plant death. The main effect of virus infection is a reduction in corm

size and quality, with yield losses of up to 20% being reported. There are currently five viruses reported to infect taro with varying distribution throughout the Pacific Islands. Dasheen mosaic virus (DsMV) is a potyvirus with flexuous, rod shaped virions, which infects both the edible and ornamental aroids spread by aphids. It is characterized by chlorotic and feathery mosaic patterns on the leaf, distortion of leaves and stunted plant growth. There is some evidence that it decreases the yield. Taro bacilliform virus (TaBV) is a badnavirus. Infection with TaBV alone is thought to result in a range of mild symptoms including stunting, mosaic and down curling of the leaf blades. However, co-infection of taro with TaBV and CBDV is thought to result in the lethal alomae disease. Colocasia bobone disease virus (CBDV) is a cytorhabdovirus**.** Alone, CBDV causes bobone disease. A complex of at least two viruses, CBDV and TaBV cause alomae disease. Symptoms first start as a feathery mosaic on the leaves; the lamina and veins become thick, the young leaves are crinkled and do not unfurl normally, while the petiole is short and manifests irregular outgrowths (galls) on its surface; therefore, the entire plant is stunted and ultimately dies. The symptoms of bobone are similar, but the leaves are more stunted and the lamina is curled up and twisted. However, complete death of entire plant does not usually occur with bobone. Taro vein chlorosis virus (TaVCV) is a nucleorhabdovirus, which causes distinctive veinal chlorosis symptoms. Taro reovirus (TaRV) has been recently discovered. It has been detected in association with other viruses, yet no symptoms have been directly attributed to TaRV infection (Revill *et al.*, 2005). The presence of taro viruses currently restricts the international movement of taro germplasm. This has serious implications since many countries are denied access to agronomically elite lines including selected traditional cultivars.

The Oomycete water mould, *Phytophthora colocasiae* is a significant pathogen as it causes taro leaf blight (TLB). The pathogen causes circular, water soaked, necrotic spots on the leaves, followed by the collapse of the plant. TLB has been present in Papua New Guinea, Federated States of Micronesia, Northern Mariana Islands, Palau and the Solomon Islands for over 50 years. An outbreak in the Solomon Islands after World War II resulted in a permanent shift in some parts of the country away from taro to sweet potato and cassava production (RIRDC, 2003). TLB disease struck American Samoa and Western Samoa in 1993-1994. Since all Samoan cultivars were susceptible to this pathogen, production was devastated. Disease resistant cultivars were not introduced until early 1997, after export from Samoa had dramatically reduced causing many millions dollars losses (FAO, 2006). In 2002 the blight reduced production sufficiently in the Morobe Province in PNG such that food aid was requested. A number of the Pacific Island countries, namely Fiji, Tonga, Cook Islands and Vanuatu are considered susceptible to an outbreak as they share the common blight-enhancing conditions of rainfall greater than 2,500 mm annually which is spread relatively evenly throughout the year (RIRDC, 2003). This shows that TLB constitutes a significant threat to food security and economy in those Pacific Island countries which do not have resistant varieties and where taro is a major staple and an export commodity.

Other taro diseases include the taro soft rot, which is caused by several species of *Pythium*, which is soil borne and attacks the roots and corm. Infected plants display wilting and chlorosis of the leaves as well as proliferation of roots at the base of the

shoot; the corm becomes soft and putrid and the plant often dies. Sclerotium rot is caused by *Sclerotium rolfsii*, which causes stunting of the plant, rotting of the corm and formation of numerous spherical sclerotia in the corm. Cladosporium leafspot is caused by *Cladosporium colocasiae* where brown spots appear on the older leaves.

Amongst the pests, taro beetle belonging to the genus *Papuana* is of great concern. The adult beetles which are black, shiny and 15-20mm in length fly from the breeding sites to the taro field and tunnel into the soil just at the base of the taro corm. They further proceed to feed on the growing corm leaving large holes that reduce the eventual market quality. Further, the wounds they create while feeding promote the attack of rot-causing organisms. The feeding activity can cause wilting and even death of the affected plant. Other insect pests include taro leafhopper (*Tarophagus proserpina*), which transmits viruses and may also cause wilting and death of the plant in heavy infestations and the sweet potato hawk-moth whose larvae defoliate the plant and the armyworm or cluster caterpillar, which also do extensive damage to the leaves. These diseases and pests are becoming a threat to taro industry in the Pacific. Thus generating substantial numbers of disease-free planting material and/or breeding resistant taro varieties are necessary.

Sexual reproduction

Taro is mainly vegetatively propagated (Shaw, 1975; Strauss *et al.*, 1979), but may also reproduce sexually (Ivancic, 1992). Due to vegetative/clonal propagation, there is almost no genetic variation within the cultivars although somatic mutations do occur thus increasing their vulnerability to pest and diseases or changes in climatic conditions (Ivancic, 1992).

Sexual hybridization of taro is well documented and techniques for pollinating and growing seedlings have been established (Wilson, 1990; Tyagi *et al.*, 2004). Sexual hybridization is one way to generate new cultivars with improved qualities (Strauss *et al.*, 1979). Extensive breeding programs (sexual propagation) have been carried out in Samoa, Papua New Guinea and Hawaii to produce cultivars with resistance to Taro Leaf Blight (TLB) and with high yields (Lebot and Aradhya, 1991; TaroGen, 1999; Singh *et al.*, 2001). From this breeding program, promising taro cultivars resistant to TLB have been produced in Hawaii (Trujillo *et al.*, 2002), PNG and Samoa (SPC, 2002b).

Even though sexual hybridization of taro is promising, it is a labor intensive and lengthy process in terms of field preparation, planting of parents, induction of flowering, pollination, development and maturation of fruit heads and seed harvesting. In addition, the germination and planting of seedlings and screening processes take several years. "It often takes 10 years or more from the time you make a pollination, until the new, improved cultivar finally reaches a large number of farmers" (Wilson, 1990). Further, viable seed production depends on the availability and compatibility of resistant germplasms as well as the vagaries of weather and pests and diseases.

Micropropagation

Intensive clonal propagation of axenic and disease-free taro through tissue culture is another option (Jackson *et al.*, 1977), which involves excising taro apical and axilary buds, decontaminating them and culturing *in vitro* in sterile nutrient medium. The cultured bud can then be grown into a plantlet, and intensive sucker production induced by the application of plant growth regulators. Tuia (1997) developed an efficient taro multiplication protocol using Murashige and Skoog (1962) medium with 30 g/L sucrose, 7.75 g/L agar and the growth regulators, TDZ and BAP. Multiplication is done in three stages: (I) 0.5 mg/L TDZ for four weeks, (II) 0.8 mg/L BAP for three weeks and (III) 0.005 mg/L TDZ for three weeks. Following multiplication, small suckers are allowed to develop into larger plantlets by first culturing individual suckers in hormone-free liquid MS medium for two to four weeks followed by culturing in agar-solidified MS medium with monthly subcultures. If the meristem is cultured rather than the whole bud, it is possible to eliminate viruses, which are particularly problematic in vegetatively propagated crops.

Protoplast culture

Regeneration of taro plants from protoplasts has been reported in *C. esculenta* var. *antiquorum* (Murakami *et al*., 1995). The frequency of regeneration has been reported to be very low and also a lengthy process. Protoplast culture has not been reported in *C. esculenta* var. *esculenta* possibly due to the lack of an efficient and routine callus initiation protocol. Regenerative callus is a prime requirement for an efficient protoplast culture (Murakami *et at*., 1995). There are no reports of attempts to improve taro through protoplast fusion.

Organogenesis and somatic embryogenesis

De novo regeneration in *Colocasia esculenta* has been reported (Yam *et al*., 1991; Thinh, 1997; Verma *et al.*, 2004; Deo, 2008). Initiation of highly regenerable callus is the first step towards an efficient regeneration system. Callus initiation protocols have been well established in *Colocasia esculenta* var. *antiquorum* (Jackson *et al.*, 1977). However, these protocols do not appear to be suitable for cultivars belonging to *Colocasia esculenta* var. *esculenta.* Researchers that have worked with *Colocasia esculenta* var. *esculenta* for many years have not reported callus formation as easily achievable (M.Taylor pers.comm.).

In a small number of cases, induction of organogenic callus in *Colocasia esculenta* var. *esculenta* has been reported. Yam *et al* (1990; 1991) reported some success using axillary buds, half-strength MS macronutrients, one tenth-strength MS micronutrients, full-strength MS vitamins, 25 ml/L taro corm extract (TE) and the plant growth regulator 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). This suggests that full-strength nutrients are not conducive to callus formation in taro. This finding has been further substantiated by the work of Deo *et al* (2009). Further, it has been suggested that TE is an important requirement for callus initiation. However, TE is an undefined component of a culture medium and so it is not possible to achieve consistency in both the combination and

concentration of components in each "individual" taro extract. In addition, the active components in the TE responsible for callus initiation have not been identified.

There are three reports of somatic embryogenesis in taro (Thinh, 1997; Verma *et al.*, 2004; Deo *et al.*, 2009). Using MS medium Verma *et al* (2004) developed a two-step protocol (initially on medium supplemented with 2.2 mg/L 2,4-D and 0.44 mg/L TDZ followed by a culture phase with 1.1 mg/L TDZ) to regenerate somatic embryos from petiole explants, with a maximum of 25-30 somatic embryos generated per explant. Thinh (1997) induced somatic embryos from the petiole fourth from the apical dome using MS medium containing 1.0-2.0 mg/L TDZ. Both reports follow direct somatic embryogenesis. Thinh (1997) stated that the somatic embryos were obtained from *C. esculenta* var. *antiquorum* however, Verma *et al* (2004) did not state whether the variety was *esculenta* or *antiquorum*. To date, there is only one report of an efficient protocol for indirect somatic embryogenesis (that is via an intervening callus and subsequent initiation of cell suspension culture from embryogenic callus) including an effective regeneration protocol for somatic embryos in *C. esculenta* var. *esculenta* (Deo *et al*., 2009) with somatic embryos formation efficiency at a rate of approximately 500-3000 per mL settled cell volume (SCV) and 80-100 per gram solid media-derived callus with embryo conversion rate into plants of approximately 60 %.

Briefly, in this protocol (Deo *et al*., 2009) corm slices derived from *in vitro* taro plants are cultured on half-strength MS medium containing 2.0 mg/L 2,4-D for 20 days in darkness followed by subculture on the same medium but containing 1.0 mg/L TDZ under the same conditions. Embryogenic callus eventuated after 75 days and continued to do so for 100 days. Using 1.0 mg/L 2,4-D and 0.5 mg/L TDZ also produced embryogenic callus but at a lower frequency than 2.0/1.0 combination. Due to differences in genotypes response to plant growth regulators, these concentrations might be more effective to other taro genotypes. Callus derived from both hormonal regimes was proliferated on half-strength MS containing TDZ (1.0 mg/L), 2,4-D (0.5 mg/L) and glutamine (800mg/L) and upon transfer to half-strength MS it differentiated into embryos which germinated on the same medium.

Cell suspension was formed by putting callus pieces (~ 0.5 g) in liquid half-strength MS medium containing TDZ (1.0 mg/L), 2,4-D (0.5 mg/L) and glutamine (100 mg/L) and agitating on a rotary shaker at 90 rpm with weekly subculture (for details see Deo, 2008) Embryo formation was induced when suspension cells were plated on half-strength MS containing TDZ (0.1 mg/L), 2,4-D (0.05 mg/L), glutamine (100 mg/L) and sucrose (50 g/L). Maturation and germination was achieved on half-strength MS containing IAA (0.1 mg/L), BAP (0.05 mg/L).

Even though various parameters were studied and optimized, a large number of embryos did not develop past the globular stage indicating that production of a large number of embryos does not always translate into a high regeneration rate. Thus, further studies are required to refine the parameters affecting embryo maturation and germination. Consequently, this regeneration system can be used for mass propagation of clean planting material.

Genetic transformation

Production of improved plant varieties via genetic transformation offers an attractive alternative to conventional breeding. Transformation of some agronomically important monocotyledonous crops such as sugar cane (Bower and Birch, 1992), banana (Becker *et al.*, 2000; Khanna *et al.*, 2004), maize (O'Kennedy *et al.*, 2001), wheat (Jones, 2005) and rice (Riaz *et al.*, 2006) has been successfully achieved using both *Agrobacterium tumefaciens* and microprojectile bombardment gene transfer methods. Genetic transformation of *Colocasia esculenta* var. *esculenta*, however has been largely neglected possibly due to the difficulties in developing an efficient regeneration system or the lack of focus on a crop of low significance to developed nations where a large portion of funding and expertise resides.

The development of a plant transformation system requires a method of transferring genes into plant cells, a gene conferring the useful new trait together with a promoter directing the appropriate level and pattern of expression, an effective selective agent to suppress the growth of non-transformed cells and the ability to regenerate plants from single transformed cells (He *et al.*, 2008). Fukino *et al* (2000) reported transformation in taro (*C. esculenta* var. *antiquorum*) callus by particle bombardment where 96 bombardments were conducted and only two putative transgenic plants were analyzed. Transformation in *Colocasia esculenta* var. *esculenta* via microprojectile bombardment (He *et al.*, 2004) and *Agrobacterium tumefaciens* (He *et al.*, 2008) has also been reported. The efficiency of transformation via biolistics was reported to be very low with only one stably transformed plant generated while a slightly higher frequency of transformation was achieved via *Agrobacterium* where six stable transgenic plants were generated.

Deo (2008) described the development of effective transformation system in the *esculenta* variety using both *Agrobacterium tumefaciens* and microprojectile bombardment of regenerable embryogenic suspension cultures. Putative stably transformed embryos expressing the *gfp* reporter gene with an efficiency of ~ 200 and ~ 17 per mL SCV were generated using microprojectile bombardment and *Agrobacterium tumefaciens*, respectively. However molecular characterization of these embryos was not done. The previous research groups (He *et al*., 2004; 2008) used regenerable callus as the target tissue for transformation which might have resulted in low frequency of transformation. Embryogenic suspension cultures are considered to be an excellent target tissue for genetic transformation since they (i) can be easily proliferated and thus provide ample target tissue, (ii) consist of small cell clumps allowing maximal exposure to the transforming agent thereby facilitating the identification of independent transformation events within the dispersed cell clusters under selection and (iii) allow the recovery of non-chimeric transformants due to the unicellular origin of embryos (Aguado-Santacruz *et al.*, 2002; Sahrawat *et al.*, 2003; ul-Haq, 2005).

Another important aspect of transgenesis is the relative activity and tissue specificity of promoters needed to control transgene expression. Transient activity of maize polyubiquitin-1 (*Ubi*-1) promoter, cauliflower mosaic virus (CaMV 35S) and taro bacilliform virus (TaBV-600) promoters has been examined in both bombarded leaves

(Yang *et al.*, 2003) and embryogenic suspension cultures (Deo, 2008) of taro. A comparison of promoters in stably transformed taro is required to fully assess and compare the strength and tissue specificity of these promoters.

Future perspectives

Extensive field trials of taro plants generated via somatic embryogenesis is required to ascertain the frequency of somaclonal variation. In addition, the vigor of plants generated by this method, in terms of their growth rate, pest and disease resistance and corm characteristics including size, quality and taste also needs to be investigated. Interestingly, banana plants derived from tissue culture are more vigorous than field-derived suckers but are more susceptible to pests and diseases (Smith *et al.*, 1998). Finally, the cost and feasibility of large-scale plant production using embryogenesis needs to be evaluated to ascertain whether this is commercially viable.

A further step along the path of using embryogenesis for mass propagation involves synthetic seeds. Synthetic seeds are somatic embryos encased in a protective coating like alginate hydrogel. When planted in a suitable medium, the coating decomposes and the somatic embryo germinates like a normal seed (Saiprasad, 2001). This would eliminate the labour costs and space requirements for embryos germinated *in vitro* and may provide a means of storing and moving germplasm. There are contrasting reports on the dormancy and viability of taro seeds produced by sexual hybridization (Strauss *et al.*, 1979; Wang, 1983) and this may also be problematic for somatic embryos.

Although it is now possible to confer many different traits through transgenics, the highest priorities for taro would be resistance to taro leaf blight and taro beetle, which are considered the most serious threats to production. Strategies for resistance to fungi such as transformation with the chitinase gene are unlikely to be effective against taro leaf blight as *Phytophthora* is not a member of the Fungi Kingdom and its cell wall is composed of cellulose rather than chitin. Other approaches for resistance to microorganisms such as antimicrobial proteins (Tripathi *et al.*, 2004) and anti-apoptotic genes (Dickman *et al.*, 2001) may be more effective. Identifying a suitable promoter driving these genes in taro leaves may be an issue requiring considerable experimentation on gene expression pattern and stability in transgenic taro. Although a preliminary promoter study has been undertaken recently (Deo, 2008), a greater range of promoters in stably transformed plants needs to be assessed in the field.

For generating transgenic taro resistant to taro beetle, a protein toxic to taro beetle but innocuous to humans and livestock would be required as well as a promoter which could drive the appropriate level of expression in corms. One potential class of proteins are protease inhibitors such as trypsin inhibitors, which are also derived from plants. These have been reported to confer resistance against pests in some crops such as tomato, potato, rice, strawberry and tobacco. These inhibitors control the growth of insect larvae by inhibiting their gut proteases (Mochizuki *et al.*, 1999; Bell *et al.*, 2001; Shukla *et al.*, 2005). Pusztai *et al* (1992) reported that the cowpea trypsin inhibitor (CpTI) does not

have serious long-term effects on the nutritional value of food. This gene, being of plant origin, would be of less concern to the public than genes derived from other organisms.

Conclusion

Although improvements in the major grain crops have increased world food production dramatically during the last twenty years, these advancements have been lacking in areas where root crops are major staples. This is largely because these crops have a lower profile in the research world, and in general do not attract as much donor funds. Much of the research currently carried out on crops is through the International Agricultural Research Centres (IARCs). Unfortunately, no IARC has the mandate to conserve and carry out research on taro. However, these staple root crops are very important to food security in many Pacific Island countries, and any research benefiting their production would be worthwhile and beneficial to the entire Pacific region. The development of somatic embryogenesis and transformation protocols provides an opportunity to significantly contribute to the mass production of disease-free planting material and to the molecular breeding of taro which to date, has been neglected. Although further refinements of these systems can be made, they are already sufficiently developed for the technology to be applied in addressing some of the limitations to taro production.

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