Genetic Improvement of Taro (*Colocasia esculenta var esculenta*) through in-vitro mutagenesis

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Abstract

Mutation breeding has been applied for many years for the improvement of crops. According to the FAO/IAEA mutant variety database, there are at least 2300 mutant varieties released officially worldwide. Taro (*Colocasia esculenta)* is vegetatively propagated, hence induced mutations offer the best way to induce variability in breeding program. *In-vitro* culture technique has also been applied to recover mutated cells through repeated *in- vitro* multiplication and to accelerate the breeding time. In the *in-vitro* culture media test, Murashige and Skoog medium supplemented with Indole –3-acetic acid *(10 mgL^{-1})* was found optimum for initiation and growing of taro. Benzylaminopurine (2 *mgL^{-1})* or Thidiazuron (0.9 *mgL^{-1})* was found optimum for multiplication of taro. In the radio-sensitivity test, shoot tips were irradiated from 0 to 60 grays of a ^{135}Co gamma irradiation source and cultured on MS Medium supplemented, with 10 mgl^{-1} Indole –3-acetic acid. The effective mutation dose (LD_{30}) that causes 30% reduction in growth was found to be 7.65 grays. 300 shoot tips were irradiated with 7.65 grays and multiplied for four generations. A population of more than 30,000 *in-vitro* plantlets of taro has been reached. A low-cost alternative substrate (60% composted scum + 40% fly ash) for hardening of tissue-cultured plantlets of taro was identified. 3500 plantlets were already hardened and used to optimize screening protocols for disease resistance against *Phytophthora colocasiae*.

Key words: taro, mutation breeding, *in-vitro* culture, Phytophthora Leaf Blight resistance.

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1. INTRODUCTION

Taro (C*olocasia esculenta (L) Schott*) is an important root crop throughout the tropics, particularly in West Africa, East and South East Asia and the Pacific Islands. It is the most important edible member of the aroid family (*Araceae*), which includes other edible members such as *Xanthosoma, Alocasia* and *Cyrstosperma*. It is thought to have originated in the Indo-Malaysian region, probably in Eastern India and Bangladesh. Cultivated types of taro are mostly diploid (2n: 2x=28), although some triploids are also found (3n: 3x = 42). There are two major taxonomic varieties, namely the dasheen type (*C. esculenta var esculenta*), with a large central corm, with suckers and stolons. The second one is the eddoe type (*C. esculenta var antiquorum*), characterized with a small central corm and a large number of smaller cormels. Taro contains abundant starch, some minerals and low-content fat in corm and is carotene rich in the leaf and petiole. The taro corm is one of the main sources of starch in many countries and together with taro leaf, petiole, and flowers can be eaten as vegetables.

Worldwide, taro ranks fourteenth among stable crops, with 9 million tons produced globally on some 2 million hectares of land, out of which 74.9% of taro is produced in Africa whereas 21.7% is produced in Asia. Taro is a significant export commodity in many countries. Japan is the major world importer of the small-corm taro, with annual quantities averaging 20,000 tons fresh and 55,000 tons frozen. China supplies most of the imports to Japan. Most Pacific Islands produce large corm taro for house and domestic consumption and for export to New Zealand, Australia and the USA.

In Mauritius, the average annual production of taro is about (92.3 tons (1993-2001). The production of taro has dropped drastically from 480 tons in 1993 to 115 tons in 2000, with a subsequent increase in its retail price in the local market. This situation was mainly due to the epidemic outbreak of Taro Leaf Blight (TLB) disease, caused by *Phytophthora colocasiae*. In fact, Taro Leaf Blight is the most destructive fungal disease of taro. It is considered to have originated in South East Asia (Trusilla 1967; Zhang *et al.,* 1994) and is widely distributed throughout the tropical regions of the World (CMI, 1997). This disease can lead to a 30-40% crop loss in heavily infected taro fields (Jackson *et al.*, 1975).

As locally available taro germplasm are susceptible to Taro Leaf Blight disease, a breeding programme using *in-vitro* mutagenesis technique was initiated with a view to develop mutants of taro showing resistance to the disease. This method of improving taro was chosen because the introduction of resistant germplasm in Mauritius is prohibited due to the embargo laid by the local quarantine services on importation of any plant from the Araceae family. This measure is important to protect the Anthurium industry from bacterial infection. Even *in-vitro* plantlets of taro are not allowed for importation. Moreover, taro being propagated mainly by vegetative means, it is difficult to obtain genetic variation within the species. Thus, mutation breeding is one of the best routes to induce genetic variability in selected genotype of taro and screened for disease resistance.

During the last five decades significant contribution has been made by several scientists in understanding the nature of mutations, development of improved methodologies for mutation induction and utilization of induced mutations in crop improvement programme. The physical mutagens like x-rays, gamma rays, fast and thermal neutrons, heavy ion beans and chemicals like Ethyl Methane-Sulphonate, Di-ethylsulphonate, Sodium Azide etc, have been used for inducing of mutations. The FAO/IAEA mutant varieties database indicates that 2252 mutant crop varieties have been officially released. These mutant varieties are selected

for increased yield, better quality and combined pest and disease resistance. Among these varieties 1585 were released as direct mutants and 667 varieties were developed using induced mutations in cross breeding (Maluszyuski *et al.,* 2000). In the database, 225 such varieties can be found and they include agricultural and horticultural crop species, not including several hundred of ornamentals. There are at least 58 mutant varieties with improved disease resistance (14 varieties of barley, 8 of bean, 6 of wheat, 6 of oats, 4 of rive, 4 of durum wheat, as well as varieties of soya, jute, mustard, cotton, peppermint, millet, sugarcane and apple. Only one mutant taro variety with improved yield was listed on the database. Thus, there is well-published evidence that mutation breeding has been used to develop disease resistant mutants among different crop varieties.

Furthermore, recent advances in tissue culture (*in-vitro)* and DNA technology offer new and existing challenges for development of new varieties. The combination of mutation breeding and *in-vitro* culture, also called *in-vitro* mutagenesis have been found to make the induction and selection of induced mutations more effective and it speeds up the production of mutants as a result of an increased propagation rate and a greater number of generations per unit time and space (Marpurgo *et al.,* 1997).

This research project for improvement of taro through *in-vitro* mutagenesis focuses on the use of modern *in-vitro* culture and molecular techniques to accelerate the breeding of taro with increased disease resistance.

In this paper the findings of the *in vitro* mutagenesis studies of taro is presented.

2. MATERIALS AND METHODS

2.1 In-vitro mutagenesis studies of taro

Plant Material

Selected plants of taro of the dasheen type (*Colocasia esculenta* var. *esculenta*) were collected and grown in open field under sprinkler irrigation at Richelieu Crop Research Station. These plants were grown until new suckers and stolon were produced. Axillary buds of developing suckers (fig 1.0) were used as source of explants for the *in-vitro* mutagenesis studies.

Fig 1.0 Axillary buds of developing suckers

***In-vitro* initiation and multiplication media test for taro**

This experiment was carried out to determine the optimum media for the *in-vitro* initiation and multiplication of taro. Murashige and Skoog(MS) medium (1962) with different concentrations of Indole –3-acetic acid (IAA) at 0, 10, 15, 20 and 25 mg L^{-1}, Thidiazuron (1-Phenyl-3-(1,2,3-thiadiazol-5-yl)urea (TDZ) at 0, 0.3, 0.6, 0.9 1.0 and 1.2 mg L^{-1} and N6-benzylaminopurine (BA) at 0, 1, 2, 3 and 4 mg L^{-1} were tested.

Developing buds from selected taro plants from germplasm collection were used as a source of shoot-tips. The buds were cleaned and rinsed under running water for 1 hour. They were then washed in a solution of benlate (0.06%) for 15 minutes and then rinsed under running tap water for another 15 minutes. They were then treated with sodium hypochlorite (2%), containing one drop of tween 20 per 100ml for 15 minutes. Finally, the buds were rinsed three times with sterile distilled water in a laminar flow cabinet.

Media were solidified with Phytagel (0.18%). pH was adjusted to 5.7 ± 0.1 before autoclaving for 15 minutes at 121°C. Explants were cultured at 23 ± 2°C under 12 hours photoperiod.

Determination of effective mutation dose (LD_{30})

This experiment was carried out to determine the appropriate mutation dose (LD_{30}), which induces a 30% reduction in the growth of treated explants. An irradiator with gamma rays as source of irradiation from a caesium-137 source was used.

Thirty shoot-tips of 3-4mm, excised from tissue culture plantlets, were irradiated with 0, 2, 4, 6, 8, 10, 12, 14, 16, 20, 40, 60 Gy. Treated explants were then cultured on MS media supplemented with IAA (10mgL^{-1}). Data on percentage survival, number of leaves and roots, length of leaves and roots and number of buds were recorded on a weekly basis for duration for eight weeks.

Evaluation of alternative local substrates for hardening of taro *in-vitro* mutated plantlets.

The objective of the study was to evaluate alternative substrates, especially by-products of the sugar cane and tea industry as media for hardening of mutated tissue cultured plantlets of taro. The *in vitro* plants were prepared for transfer to soil. The *in vitro* cultured plantlets were subjected to treatments that change them from a heterotrophic to autotrophic state, and make them withstand transfer to soil. The cultured plantlets were removed from the culture vessels, washed with tap water to remove agar, kept under low humidity and diffused sunlight. Such plantlets were not exposed to direct sunlight for the first 5 to 7 days. Plants were covered with clear plastic sheet to prevent desiccation and reduce exposure to direct sunshine. This treatment conditions the *in vitro* grown plants to adjust to low humidity and high light intensity in a gradual manner.

Ten treatments were tested against control, imported substrates, commonly used at the Food and Agricultural Research Council (FARC) which consisted of a mixture of lecca, peat and vermiculite in the ratio of 2:0.5:1. Each treatment consisted of 3 trays with 50 tissue-cultured plantlets per tray. The different combinations of substrates are shown in Table 2.0. Tissue cultured taro plantlets, which were irradiated and multiplied to M1V4 in the rooting media, were used. Roots and old leaves were trimmed before planting.

Table 2.0:Combinations of substrates used

Treatments	Combinations
A	Composted scum (100%)
B	Fly ash (100%)
C	Composted scum (60%) + Fly ash (40%)
D	Composted scum (50%) + Fly ash (50%)
E	Composted scum (50%) + Bagasse (50%)
F	Composted scum (70%) + Bagasse(30%)
G	Vermiculite (50%) + Peat (50%)
H	1/3 soil + 1/3 compost + 1/3 bagasse
I	100% peat
J	Scum 70% vermiculite 30

The following measurements were taken:

a. Percentage mortality
b. Number and length of leaves after 30 days
c. Root length after 30 days

3. RESULTS & DISCUSSION

In-vitro initiation and multiplication media test for taro

In the initiation media test with IAA at levels of 0, 10, 15, 20 and 25 mg L^{-1}, more healthy and vigorous growth in terms of highest average number of leaves and roots was obtained with IAA at 10 mg L^{-1}(Fig 2.0). There are significant differences at 5 % level in number of leaves between the different concentrations of IAA.

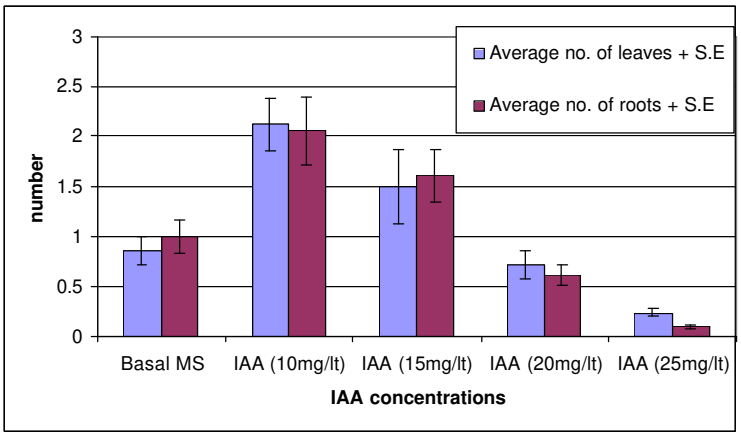
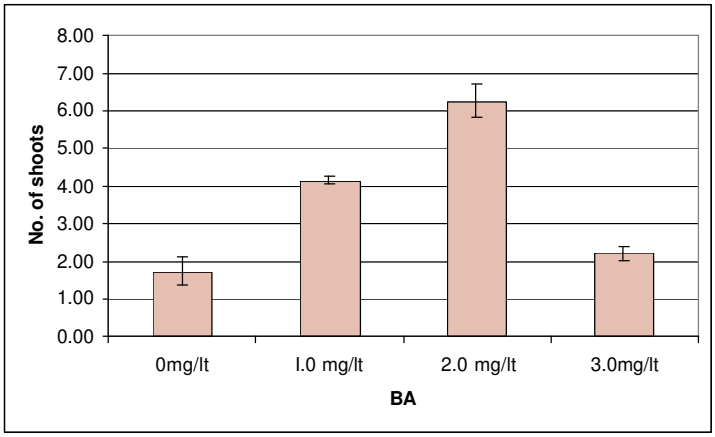


Fig 2.0: Effect of different concentrations of IAA on in-vitro initiation of taro@ 8 weeks

MS medim supplemented with IAA at concentrations above 15mg L^{-1} did not support growth of taro explants as compared to basal Murashige and Skoog medium. Growth in basal MS medium may be attributed to the effect of endogenous growth regulators. Similar observations regarding the role of endogenous levels of growth regulators in determining the shoot forming-capacity of tomato leaf disks have been reported (Kartha *et al*., 1976, Frankenberger *et al*., 1981). Another study (Elliot *et al*., 1987) has also demonstrated that a critical endogenous level of growth regulators has to be attained before cell division and organogenesis can occur.

Shoot tips of *Colocasia esculenta* var *esculenta* were cultured on MS medium supplemented with either BA at concentrations 0, 1, 2, 3 mgL^{-1} or TDZ at concentrations 0, 0.3, 0.6, 0.9, 1 and 1.2 mgL^{-1}. Highest multiplication rate of *in-vitro* shoot tips of taro was recorded in MS media supplemented with either BA at 2mgL^{-1} or TDZ at 0.9 mgL^{-1} as illustrated in Fig 3.0. However, there were no significant differences at 5 % level in number of shoot tips between 0.6,0.9 and 1 mgL^{-1} TDZ.



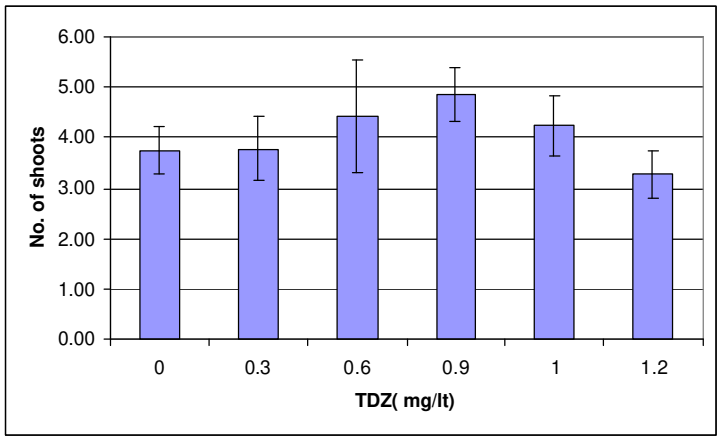


Fig 3.0: Effect of different concentrations of BA and TDZ on *in-vitro* multiplication of taro@ 8 weeks

Determination of effective mutation dose (LD_{30})

Apart from the control where the explants were not irradiated, taro shoot tips were subjected to one of eleven doses of irradiation (2, 4, 6, 8, 10, 12, 14, 16, 20, 40, 60 Gy)

The optimal dose was dependent on the parameter studied. Among all the parameters studied, number of leaves was preferred because it resulted in less experimental error than the other parameters such as survival rate, number of roots, length of leaves and roots. The effect of different doses of gamma irradiation on growth of taro plantlets is presented in Fig 4.0 and fig 5.0

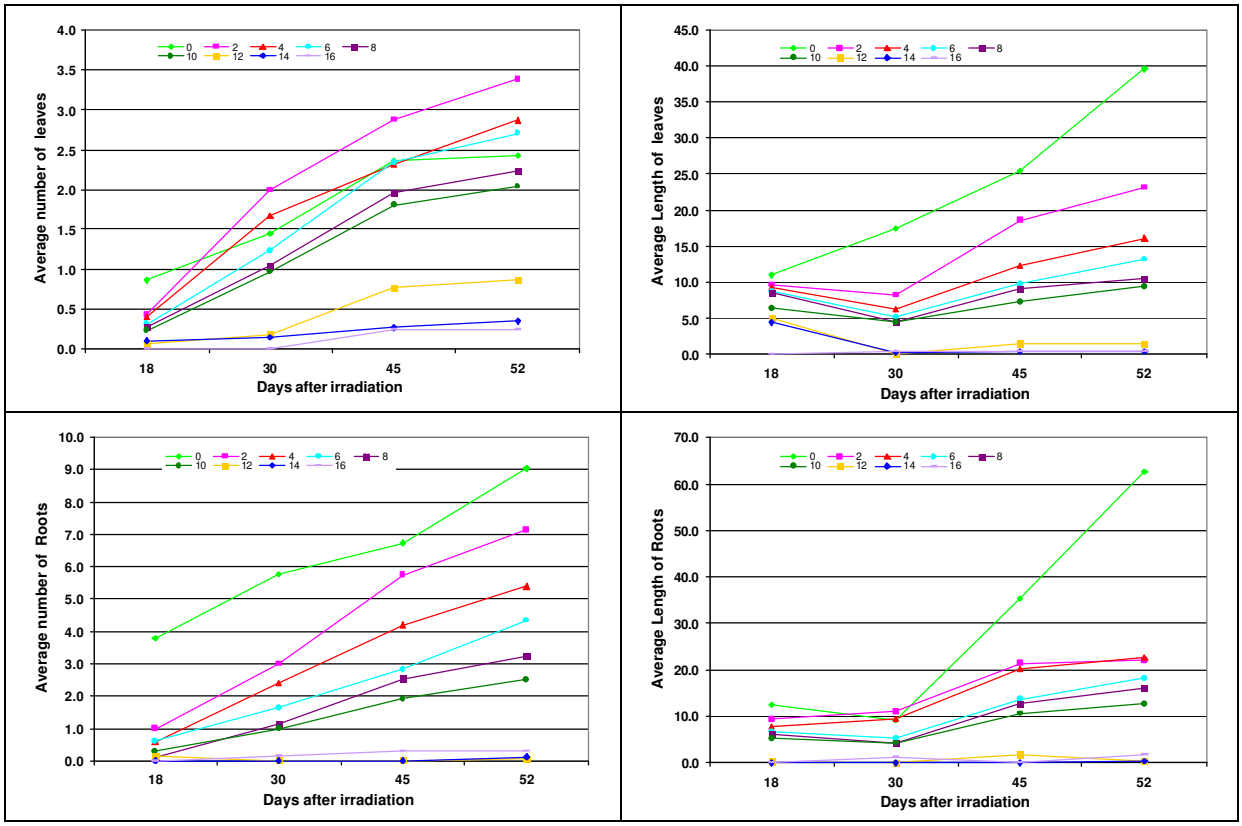


Fig 4.0 :Response of *in-vitro* culture of shoot- tips of taro after irradiation.

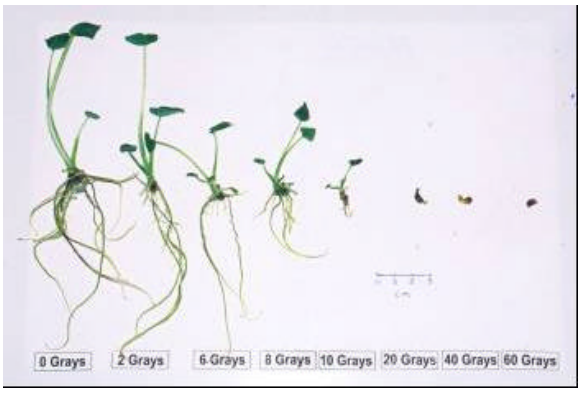


Fig 5.0: Tissue culture plantlets from shoot- tips irradiated at 0, 2, 6, 8, 10, 20, 40, 60 grays after 8 weeks in culture

The average number of leaves after 18 days of shoot-tips treated with 2 Gy was greatest, even more than that of the control indicating the boosting effect of this dose. A similar response was obtained when *Anthurium andreanum in-vitro* leaf explants were irradiated with 5 Gy. The calli and seeds also expressed better responses at the 5 Gy, but lethality at 15 Gy(Puchooa, 2005). In our study, irradiation doses above 20 Gy were lethal to taro explants. It should also be pointed out that the effective mutation dose is controlled by a number of parameters including the genotype, the type of explant, the orientation of explant on the culture medium, and the origin of the explant from the mother plant (Douglas, 1995).

Data recorded on the number of leaves showed that the effective mutation dose, which caused a 30% reduction in growth, was 7.65 Gy as shown in Fig 6.0.

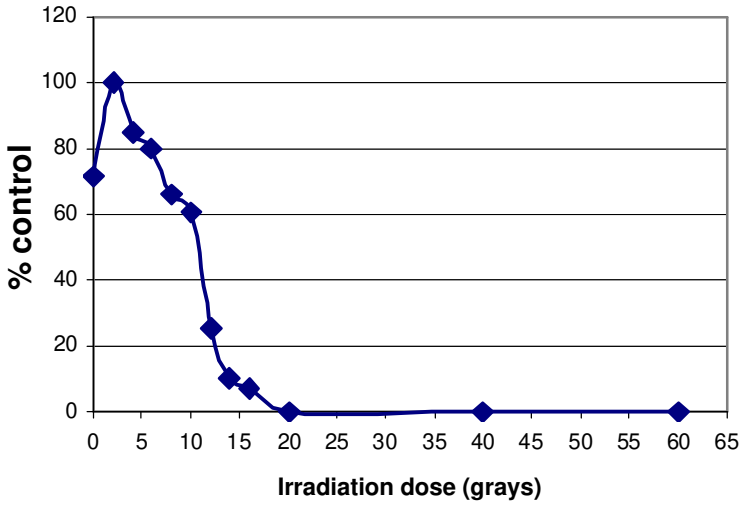


Fig 6.0: Average number of leaves 52 days after irradiation.

Evaluation of alternative local substrates for hardening of taro *in-vitro* mutated plantlets.

The Percentage mortality, number and length of leaves of hardened taro plantlets after four weeks vary with different combinations of substrate (Fig 7.0,8.0 and 9.0) Almost no mortality was recorded in three treatments, namely 50% scum+50% bagasse, 70%Scum + 30 % bagasse and100 % Peat. The number of leaves per plant from all treatments ranges from three to five. However the highest growth of leaves in terms of length, four weeks after hardening was recorded with treatments 60 % scum +40 % fly ash, followed by 50% vermiculite+ 50 % peat. Thus, in terms of cost, the combination of local available substrates 60 % scum + 40 % fly ash from sugar cane industry is selected for hardening of *in-vitro* taro plantlets. Vermiculite and Peat are indeed very expensive substrates. About 3,200 plantlets have already been successfully hardened and are being used for screening.

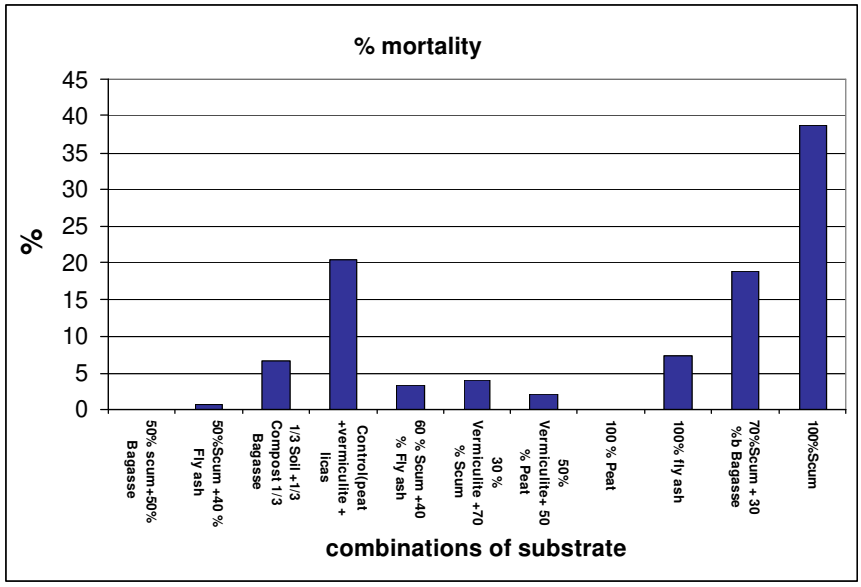


Fig 7.0: Effects of substrate combinations on % mortality of *in-vitro* taro plantlets.

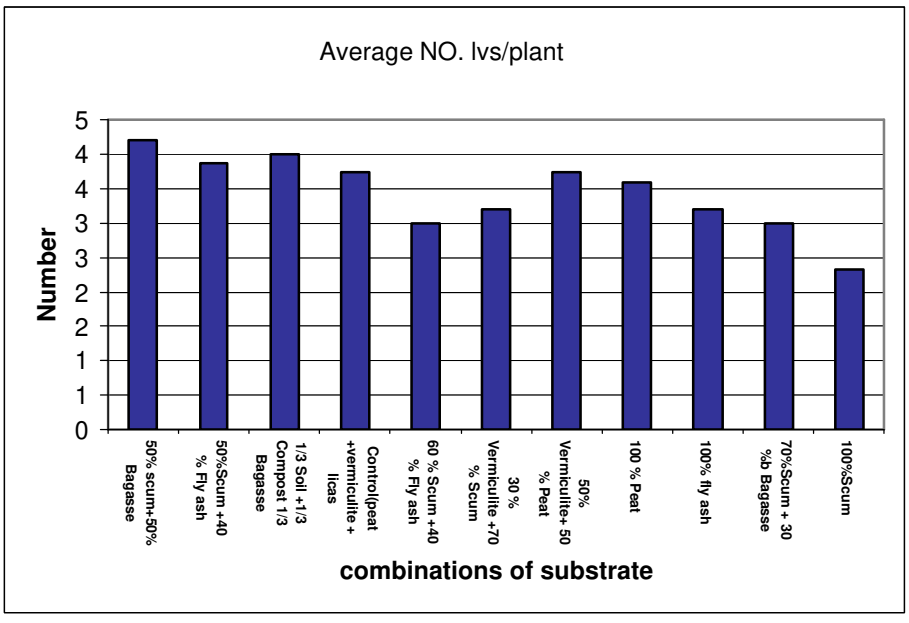


Fig 8.0: Effects of substrate combinations on No. of leaves of in-vitro taro plantlets

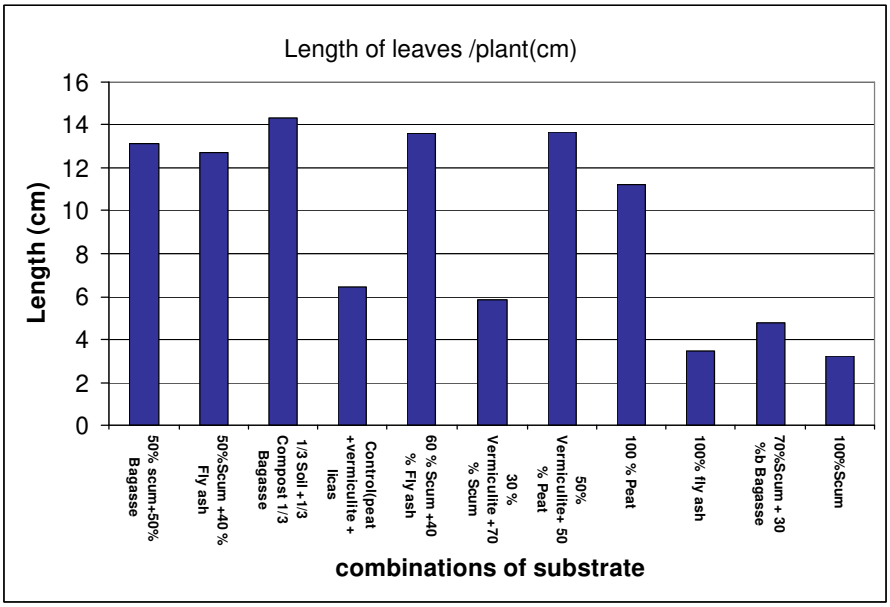


Fig 9.0: Effects of substrate combinations on length of leaves of *in-vitro* taro plantlets

Following mutagenesis and multiplication, the mutated plantlets of taro will be subjected to screening and selection for resistance against *Phytophthora colocasiae*, pathogen responsible for the colocasia leaf blight disease. Screening and selection will be carried out using under a polycarbonate house. Selected mutants of *Colocasia esculenta* var. *esculenta* will be characterized using molecular markers RAPD, SSR and IRAP.

A limitation commonly associated to the technique of *in-vitro* mutation is that the probability of achieving success is somehow aleatory. However, this intrinsic characteristic of the technique will be compensated with the use of large treated populations, and an efficient screening protocol to select mutant of disease resistance against *P.colocasiae* among the other mutants and the non-mutated tissue.

4. CONCLUSION

In this study, protocols for *in-vitro* initiation and multiplication of taro have been developed, effective mutation dose (LD_{30}) for *in-vitro* shoot tips of taro have been determined.

A large population of *in-vitro* mutants of taro, already hardened is being screened for Phytophthora leaf blight resistance and resistant genotypes will be evaluated in multilocational trials, under different disease conditions in Mauritius. Mutant germplasm will be also characterized using molecular markers of RAPD, IRAP and SSR.

Such technique of *in-vitro* mutagenesis will be further extended to other vegetatively propagated crops of economic importance in Mauritius, such as banana and anthurium.

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