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Quality and yield improvement of taro (*Colocasia esculenta* (L.) Schott) through *in vitro* mutagenesis

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Abstract

The present study was conducted to identify optimal doses of physical and chemical mutagens using *in vitro* culture techniques for an effective mutation breeding programme in taro (*Colocasia esculenta* (L.) Schott) cv. Godavari chema. Gamma irradiation was used as the physical mutagen, while ethyl methane sulphonate (EMS) and sodium azide (SA) were employed as chemical mutagens. *in vitro* grown plantlets were exposed to gamma irradiation doses ranging from 1 to 20 Gy, with an untreated control. Chemical mutagenesis was carried out using EMS at concentrations of 0.5, 1.0, 1.5, and 2.0% and sodium azide at 0.2, 0.4, 0.6, and 0.8%, each with respective untreated controls. Treated explants were inoculated onto MS medium for shoot and root regeneration.

Germination and mortality percentages were recorded. A progressive increase in mortality and a corresponding decrease in germination percentage was observed with increasing doses of both physical and chemical mutagens. Higher doses of gamma rays and chemical mutagens were found to be lethal, whereas lower doses were comparatively safer and more effective for inducing desirable variability. The lethal dose causing 50% mortality (LD₅₀) was estimated to be approximately 7 Gy for gamma irradiation. For chemical mutagens, the LD₅₀ values were determined as 1.008% for EMS and 0.351% for sodium azide, which were inferred as 1.0% and 0.4%, respectively. Survival percentage decreased with increasing mutagen dose during *in vitro* culture and hardening. Results suggested that lower doses of gamma rays, EMS and sodium azide are suitable for inducing mutations with minimal biological damage in taro.

Keywords: *Colocasia esculenta* cv. Godavari chema, EMS, gamma rays, LD₅₀, mutation breeding, *in vitro*, sodium azide

Introduction

Colocasia esculenta (L.) Schott is an important tuber crop that is widely cultivated and consumed in many developing countries, where it serves as an excellent source of carbohydrates (Ivancic, 1992) [24]. It ranks as the fourteenth most consumed vegetable worldwide and constitutes a staple food for a large population across the globe (Rao *et al.*, 2010) [41]. In Andhra Pradesh, taro is cultivated over an area of about 510 ha with an annual production of 6,630 tonnes (Anon., 2024) [4]. The corms of taro are nutritionally rich, providing approximately 112 kcal of energy, 26.46 g of carbohydrates, 0.20 g of total fat, 1.50 g of protein and 4.1 g of dietary fibre per 100 g of fresh weight (USDA National Nutrient Database 2016) [56].

Taro is a vegetatively propagated crop and its cultivation is constrained by the requirement for large quantities of planting material, high incidence of seed rotting and susceptibility to pathogenic agents. Conventional breeding in taro is therefore difficult, making induced mutation an attractive approach for crop improvement. Mutations can be induced using physical mutagens or chemical mutagens such as diethyl sulphate (DES), ethyl methane sulphonate (EMS) and sodium azide. Chemical mutagens act primarily through alkylation of DNA, leading to changes in nucleotide sequences and the induction of point mutations (Broertjes and van Harten, 1988) [9].

Advances *in vitro* culture and DNA technologies have enhanced crop improvement strategies, particularly through *in vitro* mutagenesis, which improves the efficiency of mutation induction and selection while accelerating mutant development (Morpurgo *et al.*, 1997) [35].

An important advantage of mutation breeding in vegetatively propagated crops is the ability to alter specific traits without affecting other desirable characters and the effectiveness of gamma rays in inducing useful mutations in *Colocasia* has already been demonstrated (Vasudevan & Jos, 1990) [57].

In light of these considerations, the present study was undertaken to evaluate the response of *Colocasia esculenta* cv. Godavari chema to the physical mutagen gamma irradiation and the chemical mutagens ethyl methane sulphonate (EMS) and sodium azide. The study aimed to determine the LD₅₀ values for both physical and chemical mutagens under *in vitro* conditions and to assess their effects on *in vitro* explants as well as on plant survival during hardening under *in vivo* conditions.

Materials and Methods

The research on *in vitro* mutagenesis in taro using physical and chemical mutagens, along with LD₅₀ determination, was conducted on *Colocasia esculenta* cv. Godavari chema at the Tissue Culture Laboratory, Regional Horticultural Research Station, Dr. Y.S.R. Horticultural University, Kovvur, during 2022-2023. The experiment was laid out in a Completely Randomized Design. Physical and chemical mutagens were studied separately with twelve and ten treatments, respectively, each replicated three times. Healthy *in vitro* cultured shoot tip explants were exposed to the respective mutagens, subcultured under controlled conditions and subsequently maintained and transferred to *ex vitro* conditions for further evaluation.

For physical mutagenesis, *in vitro* cultured shoot tip explants were carefully packed in ice-filled thermocol boxes to maintain temperature during transport and were exposed to gamma irradiation following the standard procedures at PJTSAU, Rajendranagar, using a Cobalt-60 gamma source. The gamma irradiation treatments comprised doses of 1 Gy, 2 Gy, 3 Gy, 4 Gy, 5 Gy, 6 Gy, 7 Gy, 10 Gy, 14 Gy, 16 Gy and 20 Gy.

For chemical mutagenesis, all procedures were carried out under sterile conditions in a laminar air flow cabinet at an optimum temperature of 27±1 °C. *in vitro* cultured explants were treated with filtered ethyl methane sulphonate (EMS) at concentrations of 0.5, 1.0, 1.5 and 2.0% and sodium azide at concentrations of 0.2, 0.4, 0.6 and 0.8% for duration of two hours. After treatment, the explants were rinsed three times with distilled water to remove residual mutagens. Special care was taken during handling of chemical mutagens due to their carcinogenic nature. The schematic representation of steps involved physical and chemical mutagenesis was presented in Plate 1 and 2.

Following treatment, the explants were subcultured on Murashige and Skoog medium and subsequently transferred to *ex vitro* conditions through hardening. An untreated control was maintained for comparison and all cultures were properly labelled according to the respective treatments and carefully observed throughout the experimental period.

Statistical analysis

The LD₅₀ value was estimated using probit analysis as described by Finney (1971) [19]. Analysis of variance (ANOVA) was performed using Indostat software to test the significance of treatment effects.

Results and Discussion

In mutation breeding, the primary objective is to improve well adapted plant varieties by inducing variability in key agronomic, yield and quality traits. The success of this approach depends on the mutation frequency, the number of plants evaluated and the efficiency of mutation induction. To avoid unnecessary loss of experimental material, radiosensitivity tests are essential for determining the LD₅₀ value, defined as the dose at which 50% of the treated material survives. In the present study, *in vitro* grown explants of the tuber crop *Colocasia esculenta* cv. Godavari chema were used to assess the effects of the physical mutagen gamma rays and the chemical mutagens ethyl methane sulphonate (EMS) and sodium azide (SA).

Germination and mortality percentages were recorded and LD₅₀ values were estimated using probit analysis based on responses of treated explants in comparison with untreated controls. The performance of the induced mutants was evaluated under *in vitro* conditions and during *ex vitro* hardening. The overall objective was to identify optimal mutagen doses capable of inducing maximum variability with minimal biological damage and acceptable survival rates.

Determination of LD₅₀ for physical mutagen (gamma irradiation)

Probit curve analysis revealed an LD₅₀ value of 6.8 Gy for gamma irradiation, which was approximated as 7 Gy (table 1) and fig. 1. The 95% confidence interval (CI) for the LD₅₀ estimate, calculated from the probit regression slope and its standard error, was 5.9-7.8 Gy, showing a low and precisely determined lethal dosage range for gamma irradiation in taro cv. Godavari chema under *in vitro* conditions. These findings were consistent with earlier reports by Seetohul *et al.* (2008) [47] in taro and Abdullah *et al.* (2018) [1] in ginger and Nurul *et al.* (2018) [36] in taro.

Table 1: Determination of LD₅₀ for gamma irradiation in taro cv. Godavari chema

Dose/concentration	Survival (%)	Mortality (%)	LD50 value
0 Gy (control)	78.67	21.33	6.8 Gy-7.0 Gy
1 Gy	84.00	16	
2 Gy	100.00	0	
3 Gy	69.33	30.67	
4 Gy	64.00	36	
5 Gy	57.33	42.67	
6 Gy	53.33	46.67	
7 Gy	49.33	50.67	
10 Gy	37.33	62.67	
14 Gy	18.67	81.33	
16 Gy	6.67	93.33	
20 Gy	0.00	100.00	

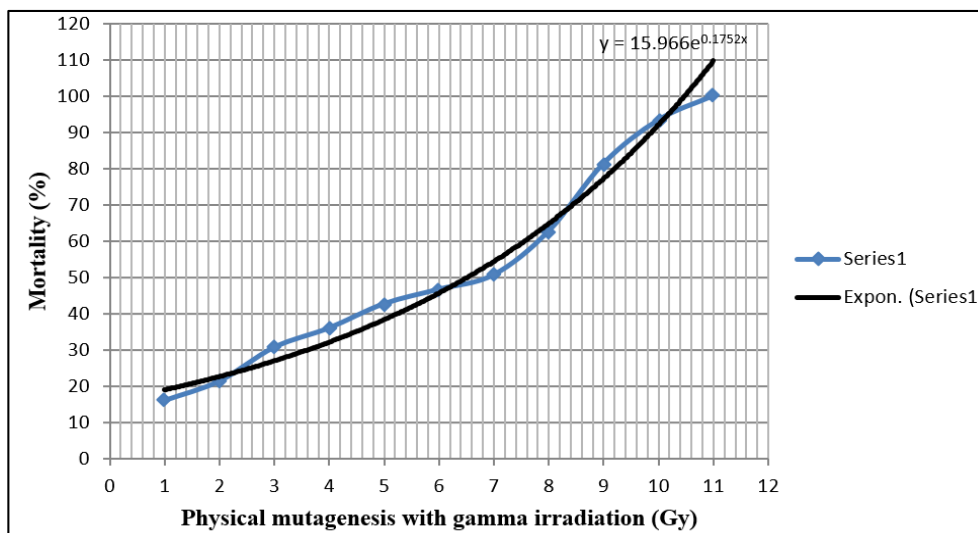


Fig 1: Determination of LD₅₀ value for gamma irradiation of *in vitro* *Colocasia* plantlets.

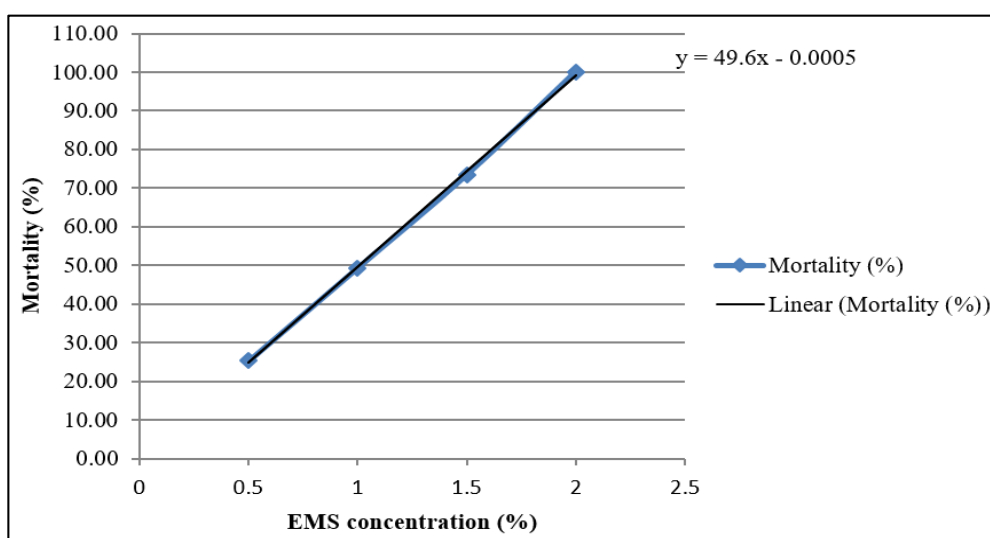
Determination of LD₅₀ for chemical mutagens (Ethyl methane sulphonate and Sodium azide)

For the chemical mutagen ethyl methane sulphonate (EMS), the LD₅₀ value was estimated at 1.008%, which was inferred as 1.0%. The 95% confidence interval for the EMS LD₅₀ was 0.87-1.15%, indicating substantial variability in the dose-response relationship due to the rather steep probit regression slope at intermediate dose concentrations. This result was in agreement with earlier findings reported in cassava by Magaia *et al.* (2015) [17], in tomato by Sikder *et al.* (2013) [48] and in pea by Dhulgande *et al.* (2011). For sodium azide, the LD₅₀ value was estimated at 0.351%, which was approximated as 0.4% (table 2). The 95% confidence interval for the sodium azide LD₅₀ ranged from 0.30% to 0.41%, being the narrowest of the three mutagen estimations, which aligns with the steep and consistent dose-response curve demonstrated by this mutagen. These findings were consistent with reports by Saddam Hussain *et*

al. (2017) [44] in *Brassica napus*, Amol *et al.* (2018) [2] in okra and Bala *et al.* (2018) [6] in tomato.

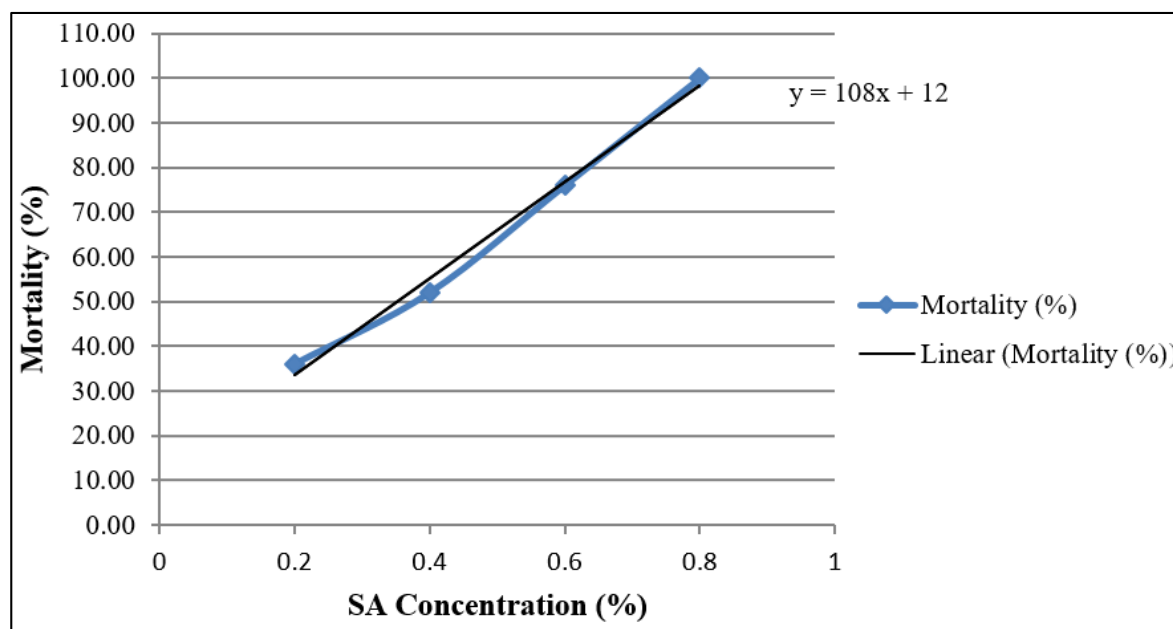
Table 2: Determination of LD₅₀ for chemical mutants EMS and Sodium Azide in cv. Godavari chema.

Treatments	Mean	LD ₅₀ value
EMS 0%	74.67	1.008%
EMS 0.5%	100.00	
EMS 1%	50.67	
EMS 1.5%	26.67	
EMS 2%	0.00	
SA 0%	64.00	0.351%
SA 0.2%	100.00	
SA 0.4%	48.00	
SA 0.6%	24.00	
SA 0.8%	0.00	
SE(m)±	3.72	
C.D. at 5%	11.06	



LD₅₀ value for EMS was calculated as x = 1.008% EMS based on linear regression equation.

Fig 2: Effect of EMS on mortality of *in vitro* culture *Colocasia* plantlets.



LD₅₀ value for sodium azide was calculated as $x = 0.351\%$ sodium azide based on linear regression equation.

Fig 3: Effect of sodium azide on mortality of *in vitro* culture *Colocasia* plantlets.

Survival percentage for physical and chemical mutagens

Under *in vitro* conditions, survival percentage among gamma irradiated shoots varied markedly with dose. The highest survival was recorded at 2 Gy (100.00%), whereas complete mortality (0% survival) was observed at 20 Gy, the untreated control of cv. Godavari chema exhibited 78.67% survival. Correspondingly, mortality percentage increased with increasing radiation dose, with the lowest mortality at 2 Gy (0%) and the highest at 20 Gy (100%) (table 1). These results were in agreement with earlier reports by Beyaz & Yildiz (2017) [8] and Fadli *et al.* (2018) [18] in taro. The reduction in shoot survival was mainly attributed to the interference of gamma rays with the metabolic and physiological activities of the shoots. Sinha & Godward (1972) [50] suggested that such a decrease in survival might have resulted from disturbances at the physiological level, coupled with chromosomal damage. The gradual increase in mortality with higher gamma doses may be attributed to radiation induced point mutations, enzyme inhibition and chromosomal aberrations, which impair respiration and energy production, thereby decreasing the survival percentage at higher doses (Kumar & Munirajappa, 2013) [32].

Under chemical mutagen treatment with ethyl methane sulphonate (EMS), the highest survival percentage was recorded at 0.5% (100%), whereas the lowest survival (26.67%) was observed at 1.5% and complete lethality occurred at 2%. A progressive reduction in survival percentage was evident with increasing EMS concentration from 0.5 to 2.0% (table 2) and fig. 2. The decline in survival was attributed to interference of the mutagen with metabolic activities of the treated shoots (Sjodin, 1962) [51] in broad bean. Disturbances at the physiological level coupled with chromosomal damage were also reported to contribute to reduced survival (Sinha & Godward, 1972) [50]. Furthermore, EMS induced disruption of enzymes involved in germination may adversely affect physiological processes, leading to reduced shoot survival (Roy Chowdhury & Tah, 2011) [43] in *Dianthus*. It was also observed that the percentage survival was decreased with

increasing EMS concentration and as reported by Raina *et al.* (2022) [38], this concentration response pattern was attributed to the tendency of higher mutagen concentrations to increase mutation frequency while simultaneously intensifying biological damage, including lethality and chromosomal abnormalities.

Sodium azide, as a chemical mutagen, exhibited a similar dose dependent effect on survival. The highest survival percentage was recorded at 0.2%, while the lowest survival was observed at 0.6% and complete lethality occurred at 0.8%, which was therefore considered the lethal dose. In contrast, untreated shoots exhibited 64.00% survival (table 2) and fig. 3. These results were consistent with the findings of El-Sayed *et al.* (2012) [16] in banana, who reported a progressive decline in plant survival with increasing concentrations of sodium azide. Overall, the results indicated that higher concentrations of gamma rays and chemical mutagens such as EMS and sodium azide were lethal, whereas lower concentrations were comparatively safer and more suitable for inducing desirable mutations. Soniya *et al.* (2001) [52] also reported that the variations observed in tomato tissue culture plants treated with lower concentrations of sodium azide were due to either epigenetic changes or genetic alterations occurring in vegetative cells during differentiation and these changes were capable of producing stable plants with useful heritable traits.

Abnormalities

In the present study, a few foliage abnormalities were observed among the mutagen treated taro mutants. Yellowing of leaves was the most prominent abnormality and chlorophyll mutants were also recorded, with the frequency of chlorophyll mutations showing a dose dependent response to mutagenic treatments in *Colocasia*. Abnormalities such as partial tissue death, leaf yellowing and loss of chlorophyll were predominantly observed in chemically treated cultures, whereas such symptoms were not evident in gamma irradiated cultures (table 3). Leaf abnormalities, including elongated and deformed leaves, were observed at specific concentrations of chemical

mutagens, namely 1.0% and 1.5% EMS and 0.4% and 0.6% sodium azide (table 4). However, these abnormalities did not show a significant relationship with the applied mutagen doses. Similar foliage abnormalities induced by mutagenic treatments have been reported in *Delphinium malabaricum* (Kolar *et al.*, 2015) [31], gladiolus (Dogra *et al.*, 2017) [15], tuberose (Kayalvizhi *et al.*, 2017, Singh & Sadhukhan, 2019) [27, 49], tulips (Sedaghathoor & Sharifi 2017) [46], and chilli (Khan *et al.*, 2009) [29]. Comparable abnormalities were also observed in mutation studies conducted on eggplant (Ando & Montalvan, 2001) [3], supporting the findings of the present investigation.

Rooting and shooting characters influenced by physical and chemical mutagens

Under *in vitro* conditions, physical mutagenesis in shoots of *Colocasia esculenta* cv. Godavari chema showed a clear dose dependent effect on rooting. The maximum rooting

percentage was recorded at 2 Gy (88%) which was on par with 1 Gy (80%), whereas the lowest rooting percentage (6.67%) was observed at 16 Gy. No plant survival was recorded at 20 Gy, which was therefore considered the lethal dose (table 3). Gamma rays were known to consist of shorter wavelength electromagnetic waves, typically shorter than those of X-rays. With frequencies above 30 exahertz (3×10^{19} Hz) and wavelengths less than 10 picometers (1×10^{-11} m), gamma ray photons possessed the highest photon energy among all forms of electromagnetic radiation. When the electromagnetic gamma radiation from oscillating electric fields was absorbed by plant tissues, it caused rotation of water molecules, which led to localized heating (Khalafallah & Sallam, 2009) [28]. The excessive energy absorption at higher doses was found to disturb cellular metabolism and enzyme activity, resulting in tissue damage and a reduction in rooting percentage (Diprose, 2001) [14].

Table 3: Effect of Gamma irradiation on *in vitro* rooting of taro cv. Godavari chema

Treatments	Any Abnormalities	Percent rooting of treated cultures (%)	Number of roots per shoot	Average root length (cm)	Average shoot length at rooting (cm)
0 Gy	0	70.67	6.87	1.49	3.01
1 Gy	0	80.00	7.53	1.55	3.17
2 Gy	0	88.00	7.93	1.78	3.47
3 Gy	0	61.33	6.47	1.34	2.86
4 Gy	0	57.78	6.13	1.28	2.70
5 Gy	0	52.55	5.73	1.19	2.53
6 Gy	0	48.45	5.40	1.07	2.40
7 Gy	0	37.43	5.13	1.09	2.26
10 Gy	0	28.00	4.13	1.03	2.17
14 Gy	0	12.00	3.64	1.37	2.14
16 Gy	0	6.67	3.00	1.35	2.05
20 Gy	0	0.00	0.00	0.00	0.00
SE(m)±	0	3.36	0.21	0.08	0.07
C.D. at 5%	0	9.85	0.61	0.23	0.21

The number of roots and root length in gamma irradiated shoots followed a similar dose dependent pattern. The highest number of roots and greatest root length were recorded at the lower dose of 2 Gy (7.93 and 1.78 cm, respectively) which was on par with 1 Gy (7.53 and 1.55 cm, respectively) whereas the minimum number of roots was observed at 16 Gy (3.00) and the shortest root length at 10 Gy (1.03 cm) (table 3). A reduction in root number at higher doses of gamma irradiation has also been reported by Tiwari *et al.* (2010) [55] in gladiolus. These changes were attributed to gamma irradiation, which caused physiological disturbances affecting photosynthesis and damage to the root system, thereby hampering plant growth under *in vitro* conditions, as reported by Grabowska & Mynett (1970) [22]

in gladiolus.

Under chemical mutagenesis, rooting percentage of *in vitro* cultures decreased progressively with increasing mutagen concentration. In ethyl methane sulphonate (EMS) treatments, rooting was highest at 0.5% (88.00%) and declined to 22.67% at 1.5%, while no plantlets survived at 2.0%. In sodium azide treatments, rooting percentage decreased from 84% at 0.2% to 20% at 0.6%, with complete lethality observed at 0.8%. These findings were in agreement with Wi *et al.* (2007) [60], who reported that lower mutagen doses induced limited and beneficial physiological changes that supported normal growth, whereas higher doses caused growth inhibition due to cell cycle arrest during the rooting phase and extensive genomic damage.

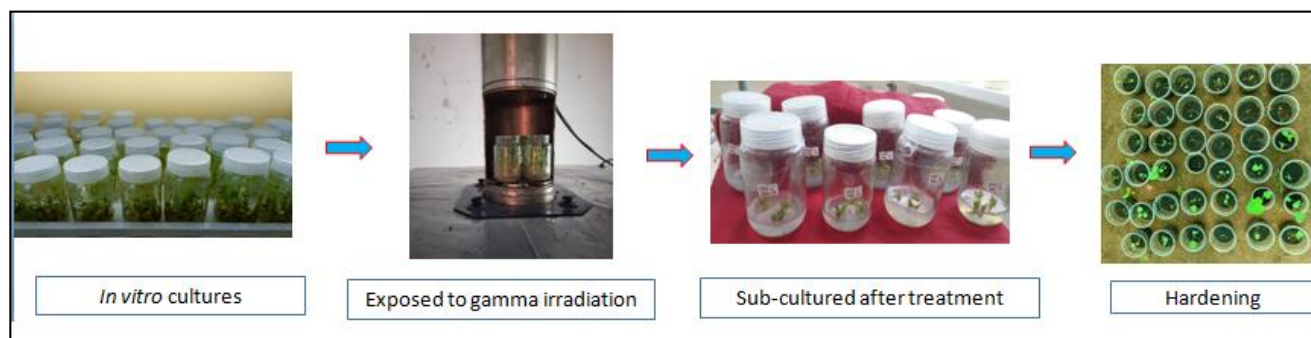


Plate 1: Steps involved in gamma irradiation of *in vitro* cultures and subsequent hardening

The highest rooting percentage was obtained at 0.5% EMS, which was attributed to the stimulatory effect of ethyl methane sulphonate at lower concentrations. Similarly, Soniya *et al.* (2001) ^[52] reported that epigenetic or genetic changes induced during tissue culture were capable of producing stable and heritable traits. It was suggested that

lower mutagen concentrations induced subtle heritable modifications, whereas higher concentrations caused more pronounced alterations and therefore the rooting variations observed in the present study were considered to have resulted from such mutagen induced changes.

Table 4: Effect of chemical mutagens on *in vitro* rooting of taro cv. Godavari chema

Treatments	Any abnormalities	Percent rooting of treated cultures (%)	No. of roots per shoot	Average root length (cm)	Average shoot length at rooting (cm)
EMS 0%	0	70.67	6.47	1.17	2.81
EMS 0.5%	0	88.00	8.40	1.82	3.49
EMS 1%	0.67	44.00	5.60	1.01	2.77
EMS 1.5%	1.00	22.67	5.47	0.95	2.31
EMS 2%	0	0.00	0.00	0.00	0.00
SA 0%	0	56.00	6.53	1.05	2.79
SA 0.2%	0	84.00	8.87	1.73	3.63
SA 0.4%	0.33	38.67	5.87	0.94	2.73
SA 0.6%	0.50	20.00	5.20	0.83	2.37
SA 0.8%	0	0.00	0.00	0.00	0.00
SE(m)±	0.25	3.43	0.45	0.09	0.10
C.D. at 5%	NS	10.18	1.33	0.26	0.28

Among the EMS treated cultures, the number of roots per shoot varied from 8.40 to 5.47, showing a decreasing trend with increasing mutagen concentration and no plantlets survived at the highest concentration of 2.0%. Similar reductions in root number following EMS treatment have been reported in rice by Talebi *et al.* (2012) ^[54], Rajarajan *et al.* (2016) ^[39] and Ramachander *et al.* (2015) ^[40]. This reduction may be attributed to the action of alkylating agents, which induce mutations by adding methyl or ethyl groups to DNA nucleotides, resulting in modified base formation. In sodium azide treatments, lower concentration

treated shoots recorded an average of 8.87 roots while the lowest number of roots was recorded at concentration 0.6% (5.20) (table 4). The reduction in root number under sodium azide treatment was explained by Kumar & Yadav (2010) ^[33] in sesame, who reported that lower mutagen doses induced only slight genetic modifications without markedly affecting growth regulator activity, whereas higher doses caused chromosomal aberrations and disrupted the production or distribution of growth regulators, resulting in reduced root number across generations.

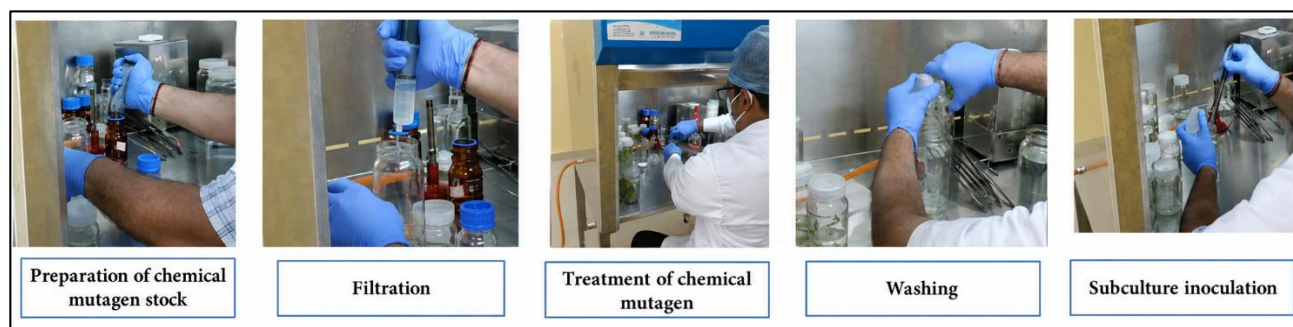


Plate 2: Steps involved in chemical mutagen treatment of *in vitro* cultures

Among the chemical mutagen treatments, EMS at 0.5% produced the longest root length (1.82 cm) which was on par with sodium azide at 0.2% (1.73 cm) while the shortest root length was recorded at 0.6% sodium azide (0.83 cm). No plantlets survived at the higher concentrations of EMS (2.0%) and sodium azide (0.8%) (table 4).

Shoot length was significantly influenced by both physical and chemical mutagen treatments. Under gamma irradiation, the highest shoot length was recorded in the treatment dose 2 Gy (3.47 cm), followed by plants treated with 1 Gy (3.17 cm), whereas the shortest shoot length was observed at 16 Gy (2.05 cm), no plants survived at 20 Gy (table 3).

The reduced shoot growth observed after mutagenic treatments might have been caused by the destruction of auxin or the inhibition of its synthesis reported by Gorden (1954) ^[21]. Similarly, Cherry & Lessman (1967) ^[11]

explained that the reduction in growth in maize might have resulted from failure of the assimilatory mechanism and changes in the specific activity of enzymes. These results were found to be in agreement with the findings of Tiwari *et al.* (2010) ^[55] in gladiolus, who reported that lower doses of gamma irradiation stimulated shoot elongation through enhanced auxin synthesis, whereas higher doses inhibited growth due to radiation induced cellular damage and suppression of auxin synthesis. The reduction in shoot length and delayed growth at higher doses were attributed to physiological disturbances and chromosomal damage caused by irradiation.

Under chemical mutagen treatments, the maximum average shoot length was recorded in the lower concentration of mutagens i.e., 0.2% sodium azide (3.63 cm) and 0.5% EMS (3.49 cm). In contrast, the minimum shoot length was

observed at 1.5% EMS (2.31 cm) followed by 0.6% sodium azide (2.37 cm), with complete lethality at higher concentrations (table 4). Higher sodium azide concentrations exerted deleterious physiological and molecular effects, possibly due to chromosomal damage, resulting in reduced shoot growth. Similar effects of chemical mutagens on shoot length have been reported in chrysanthemum cv. Maghi (Kapadiya *et al.*, 2014) [26] and gerbera (Ghani *et al.*, 2013) [20].

The above results were found to be in accordance with earlier reports on the effects of chemical mutagens on shoot growth in various crops. Yoosumran *et al.* (2010) [61] reported that increasing concentrations of EMS caused a gradual reduction in shoot length in chrysanthemum, indicating that higher mutagen concentrations suppressed shoot elongation. Similarly, Samala *et al.* (2014) [45] observed that shoot length was reduced in EMS treated chrysanthemum cultures and this effect was attributed to growth inhibition caused by mutagen induced cellular damage. In addition, Behera *et al.* (2012) [7] reported in *Aster* that higher EMS concentrations resulted in shorter shoots, suggesting that EMS interfered with normal cell division and elongation. Together, these earlier findings supported the observation in the present study that EMS decreased the average shoot length as its concentration increased.

Percent survival in hardening

Plantlets were transferred from the *in vitro* culture environment to the acclimatization medium for hardening. The irradiated corms were subcultured in rooting medium and subsequently acclimatized under net house conditions. The cultured corms were initially placed under shade for 3 to 4 days before being transferred for hardening. They were later transferred to potting media filled in plastic cups containing a mixture of red earth, FYM and sand in the ratio of 2:1:1. In this medium, physical parameters such as aeration and bulk density, along with chemical parameters like nutrient and moisture holding capacity, were assumed to be within the optimum range for the initial establishment of *in vitro* grown plantlets. Lalitha *et al.* (2023) [34] reported that in banana cv. Grand Naine, better growth was achieved in potting mixtures prepared with red earth, FYM and sand compared to other planting materials.

Survival during hardening was highest in plantlets with gamma irradiation dosage 2 Gy (100%), followed by those treated with 1 Gy (82.67%), while the lowest survival (12.00%) was recorded at 14 Gy, no plantlets survived at 16 Gy and 20 Gy (table 5). In chemical mutagen treatments, low concentration treated cultures of both EMS and sodium azide exhibited 100% survival during hardening. Among the treated cultures, the highest survival was observed at lower concentrations, namely 0.5% EMS (100%) and 0.2% sodium azide (100%), whereas the lowest survival was recorded at higher concentrations of 1.5% EMS (25.33%) and 0.6% sodium azide (22.67%) (table 6). These results were found to be consistent with the reports of Suman & Kumar (2015) [53] and Waman *et al.* (2015) [59] in banana. Waman *et al.* (2015) [59] further reported that higher mutagen concentrations caused severe cellular and metabolic damage, which accounted for the reduced survival of plantlets during acclimatization at elevated concentrations.

Table 5: Effect of gamma irradiation on survival of tissue culture plants of taro cv. Godavari chema on *ex vitro* hardening

Treatments	Survival percent from rooted cultures (%)
0 Gy	73.33
1 Gy	82.67
2 Gy	100.00
3 Gy	65.33
4 Gy	54.67
5 Gy	52.00
6 Gy	48.00
7 Gy	44.00
10 Gy	29.33
14 Gy	12.00
16 Gy	0.00
20 Gy	0.00
SE(m)±	2.37
C.D. at 5%	6.97

The contrasting LD₅₀ values obtained for gamma irradiation (7 Gy), EMS (1.0%) and sodium azide (0.4%) can be attributed to their distinct mechanisms of action and the cytological characteristics of taro. *Colocasia esculenta* is predominantly diploid (2n = 2x = 28) with a relatively large genome, which may provide partial buffering against mutagen-induced damage (Das & Das, 2014; Chair *et al.*, 2016) [10, 12]. Gamma irradiation primarily induces DNA damage through reactive oxygen species, causing strand breaks and chromosomal aberrations (Beyaz & Yildiz, 2017) [8]. Its comparatively higher LD₅₀ indicates that meristematic cells can repair a substantial proportion of radiation-induced damage through non-homologous end joining and homologous recombination (Waterworth *et al.*, 2011) [58]. In contrast, EMS acts as an alkylating agent, producing mainly G→A transitions through DNA alkylation, with cytotoxicity occurring when repair mechanisms are exceeded (Rasheed *et al.*, 2011; Arisha *et al.*, 2014) [5, 42].

Table 6: Effect of chemical mutagens on survival of tissue culture plants of taro cv. Godavari chema on *ex vitro* hardening

Treatments	Survival percent from rooted cultures (%)
EMS 0%	70.67
EMS 0.5%	100.00
EMS 1%	48.00
EMS 1.5%	25.33
EMS 2%	0.00
SA 0%	61.33
SA 0.2%	100.00
SA 0.4%	46.67
SA 0.6%	22.67
SA 0.8%	0.00
SE(m)±	3.07
C.D. at 5%	9.12

Sodium azide exhibited the lowest LD₅₀ because, besides inducing DNA lesions through its metabolite L-azidoalanine, it also inhibits mitochondrial respiration, leading to ATP depletion and enhanced cytotoxicity (Kleinhofs *et al.*, 1978; Gruszka *et al.*, 2012) [23, 30]. The multicellular nature of shoot tip explants may also contribute to the higher tolerance of gamma irradiation through chimeric survival, whereas chemical mutagens diffuse uniformly, producing a more homogeneous cellular response (Predieri, 2001; Jankowicz & Till, 2022) [25, 37]. These findings indicate that gamma irradiation is suitable

for inducing chromosomal alterations, EMS for generating point mutations with fewer chromosomal abnormalities and sodium azide should be used below its LD₅₀ to balance mutagenic efficiency and explant survival.

Conclusion

The present study demonstrated that *in vitro* mutagenesis using physical and chemical mutagens was effective in inducing variability in taro (*Colocasia esculenta* cv. Godavari chema). Survival and growth responses showed a clear dose dependent trend, with higher doses of gamma rays, EMS and sodium azide causing severe growth inhibition and lethality. The LD₅₀ values were identified as approximately 7 Gy for gamma irradiation, 1.0% for EMS and 0.4% for sodium azide, indicating their optimal ranges for mutation induction. Lower doses promoted better survival, rooting and shoot growth both *in vitro* and during *ex vitro* hardening. Higher doses induced physiological abnormalities and reduced regeneration efficiency. Overall, low dose *in vitro* mutagenesis was found to be a suitable and efficient approach for inducing desirable variability in taro with minimal biological damage.

Credit authorship contribution

- G. Komali Joseph: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing-original draft.
- K. Mamatha: Supervision, Conceptualization, Validation, Writing-review & editing.
- E. Padma: Methodology, Supervision, Writing-review & editing.
- K. Ravindra Kumar: Validation, Resources, Writing-review & editing.
- M. Girija Rani: Formal analysis, Validation, Writing-review & editing.
- D. R. Salomi Suneetha: Supervision, Project administration, Writing-review & editing

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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