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Drought tolerance of *Stylosanthes guianensis* CIAT 184 by tissue culture

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Abstract The multiple shoots induction of *Stylosanthes guianensis* CIAT 184 was cultured on MS medium supplemented with 3 mg/l TDZ. The percentage of shoot induction was 100%, the average number of shoots was 36.80 per seed, and the average height of shoots was 0.76 cm. The shoots were transferred to MS medium for rooting, supplemented with 0.3 mg/l IAA. The percentage of root induction was 40%, and the average number of roots was 6.50 per shoot. Physical gamma rays induced the mutation. Seeds were irradiated with 0, 5, 10, 15, 20, 25, 30 and 50 Krad. The results showed that *S. guianensis* CIAT 184 irradiated at 44.38 Krad gave 50 % ($GR_{50(30)}$) and 50% $LD_{50(30)}$ of 38.06 Krad. The drought tolerance of seeds supplemented with 0, 5, 10, 15 and 20% of PEG-6000. The GR_{50} was 17.73 % at 2 weeks, and the LD_{50} was 14.86 % and 11.83 % for 4 and 12 weeks, respectively.

Keywords: Drought tolerance, Mutation, Regeneration, *Stylosanthes guianensis* CIAT 184

Introduction

Thailand's agriculture depends heavily on crop cultivation and livestock, including cattle, buffalo, and pigs. However, recurring droughts during the hot, dry season increasingly deplete natural water sources, reducing agricultural productivity—especially in cattle and buffalo farming, where shortages of high-quality forage are common for small-scale farmers. In contrast, pig and poultry production are less affected because feed can be stored. With droughts becoming more severe each year, developing drought-tolerant forage crops is essential to ensure a year-round feed supply and sustain ruminant production. To address this need, the Department of Livestock Development promotes forage grasses and legumes by expanding seed production and transferring technologies for their establishment, management, and utilization.

Stylosanthes guianensis accession CIAT 184 (Stylo Thapra or Stylo CIAT 184) is a well-established tropical forage legume that has been developed into commercial cultivars in several countries and widely adopted across tropical

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America, Africa, Southeast Asia, and China (Schultze-Kraft *et al*., 2023). In southern China, it has given rise to five cultivars. It is particularly valued for its durable tolerance to anthracnose (*Colletotrichum gloeosporioides*), adaptation to acidic and infertile soils, drought tolerance, and high production of nutritious dry matter suitable for ruminants and monogastrics. In Thailand, *S. guianensis* CIAT 184 produces 8.2 and 4.4 metric tons dry matter (DM) per hectare at 60 and 75 day cutting intervals, respectively (Kiyothong *et al*., 2002), and consistently outperforms other forage legumes such as alfalfa and *S. hamata* in plant height, total biomass, and fresh and dry weight (Rupitak and Srisaikham, 2021). It also contains higher levels of structural fiber fractions, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), which are essential indicators of forage quality (Rupitak and Srisaikham, 2021). Due to its agronomic significance and inherent stress tolerance, *S. guianensis* CIAT 184 is well-suited for tissue culture and mutagenesis approaches to enhance drought tolerance.

Although *S. guianensis* CIAT 184 has many agronomic advantages, its productivity remains constrained by drought stress, a challenge expected to intensify under climate change. Enhancing drought tolerance has therefore become a key breeding goal. Combining tissue culture with gamma irradiation, a physical mutagen, offers an effective strategy to induce genetic variation and facilitate the selection of superior genotypes with improved drought tolerance. According to Pandit *et al*. (2021), physical mutagens are more commonly used in mutation breeding (2,635 cases) than chemical mutagens (398 cases). Gamma irradiation is the most widely applied among physical mutagens, accounting for 1,702 of the 2,635 reported cases (64.6%). One thousand six hundred two mutant varieties have been released in cereals, 501 in legumes, and 86 in oilseed crops. Examples of legumes include *Glycine max* L. (soybean), *Arachis hypogaea* L. (groundnut), and *Phaseolus vulgaris* L. (common bean). However, reports on mutant varieties of *Stylosanthes* remain very limited, with only a single study by Ngoenngam *et al*. (2019) documenting the in vitro effects of gamma irradiation and plant growth regulators on the induction and development of *S. hamata* cv. Verano. This study investigated the drought tolerance of S. guianensis CIAT 184 using tissue culture techniques combined with acute gamma irradiation. The objectives were to evaluate shoot and root induction, callus formation, and the median lethal dose (LD_{50}), as well as to assess the ability of seeds and callus to survive and regenerate into plantlets under drought stress.

Materials and methods

Seed sterilization

Seed material of *S. guianensis* CIAT 184, kindly provided by the Animal Feed and Forage Crops Research Group, Feed Division, Department of Livestock Development, Pathum Thani Province, consisted of mature and healthy seeds, which were selected for surface sterilization before in vitro culture. Seeds were immersed in 70% (v/v) ethanol for 2 min, then rinsed in sterile distilled water containing 1–2 drops of a surfactant. They were treated with 15% (v/v) commercial Clorox® under gentle continuous agitation for 15 min. Sterilized seeds were rinsed 3 times with sterile distilled water (5 min each rinse) and air-dried under aseptic conditions before culture.

Optimization of culture medium for multiple shoot induction

Sterilized seeds were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose, 2.6 g L^{-1} gellan gum, and adjusted to pH 5.8. The medium was enriched with cytokinins, 6-benzyladenine (BA), meta-topolin (mT), or thidiazuron (TDZ), at 0.5, 1.0, or 3.0 mg L^{-1}. Cultures were maintained under a 16-h photoperiod at 25 ± 2 °C and subcultured every 4 weeks. Data were recorded biweekly to assess shoot induction frequency (%), number of shoots per explant, and shoot length.

Optimization of culture medium for root induction

Shoots from seedling cultures were excised and transferred to MS medium (Murashige and Skoog, 1962) containing 30 g L^{-1} sucrose and 2.6 g L^{-1} gellan gum, adjusted to pH 5.8, and supplemented with auxins, IAA, IBA, or NAA, at 0.1, 0.3, or 0.5 mg L^{-1}. Cultures were maintained under a 16-h photoperiod at 25 ± 2 °C and subcultured every 4 weeks. Observations were recorded biweekly to assess root induction frequency (%), number of roots per shoot, root length, and root morphology.

Effect of gamma irradiation on seed growth

Mature seeds of *S. guianensis* CIAT 184 were exposed to acute gamma irradiation at doses of 0, 5, 10, 15, 20, 30, and 50 kilorad (krad) using a Mark I gamma irradiator with a Cesium-137 source at the Gamma Irradiation Service and Nuclear Research Center, Kasetsart University, Bangkok. After irradiation, seeds were aseptically cultured on MS medium devoid of plant growth regulators, containing 30 g L^{-1} sucrose and 2.6 g L^{-1} gellan gum, and adjusted to pH 5.8. Cultures were incubated under a 16-h photoperiod at 25 ± 2 °C and subcultured every 4 weeks. Seed germination percentage, shoot and root length,

and overall seedling vigor were recorded at two-week intervals. Data were expressed relative to non-irradiated controls to calculate the median lethal dose (LD_{50}), defined as the gamma dose at which 50 % of seedlings failed to survive within 30 days. Seedlings were considered viable when they remained green and developed shoots and roots, whereas non-viable seedlings displayed chlorosis and ultimately died. In addition, the gamma dose causing a 50 % growth reduction (GR_{50}) was determined by measuring shoot and root elongation in seedlings cultured on MS medium supplemented with 3 mg L^{-1} TDZ. Only seedlings exhibiting both shoot and root formation were included in growth measurements; seedlings developing a single organ were excluded from analysis.

Assessment of seed drought tolerance

Surface-sterilized seeds of *S. guianensis* CIAT 184 were cultured on MS medium supplemented with 0, 5, 10, 15, or 20 % polyethylene glycol (PEG 6000) to impose osmotic stress, along with 30 g L^{-1} sucrose and cotton support for seedling anchorage, and adjusted to pH 5.8. Cultures were maintained under a 16-h photoperiod at 25 ± 2 °C, and the medium was renewed every four weeks. Seed germination (%) and shoot and root lengths were recorded using a vernier caliper and expressed relative to non-stressed control seeds. The median lethal concentration (LD_{50}) was evaluated at 4 and 12 weeks based on seedling viability, where seedlings were considered alive if green with well-developed shoots and roots; seedlings that turned yellow and failed to develop were recorded as non-viable. Furthermore, the PEG concentration causing 50 % growth inhibition (GR_{50}) was determined after 2 weeks, considering only seedlings that developed both shoots and roots. This experiment also established the maximum PEG concentration that seeds could tolerate under in vitro drought stress conditions.

Statistical analysis

All experiments were arranged in a completely randomized design (CRD) with two or three replications, depending on the experiment, and an appropriate number of seeds or plants per replicate. Experimental data were analyzed using analysis of variance (ANOVA). Mean comparisons were performed using Duncan's multiple range test at a significance level of 0.05. All statistical analyses were conducted using IBM SPSS Statistics 21.0.

Results

Effect of growth regulators on shoot induction

After 5–7 days of culture, *S. guianensis* CIAT 184 seeds began germinating and producing seedlings (Figure 1A). The three tested plant growth regulators induced distinct morphogenic responses by the second week. MS medium supplemented with 0.5, 1.0, or 3.0 mg L^{-1} of BA or mT produced elongated and slender shoots, accompanied by callus formation at the radicle region. In contrast, MS medium containing TDZ at all concentrations induced de novo shoot formation from the radicle by the 2 weeks (Figure 1B-C) and multiple shoots regenerated from callus after 8 weeks (Figure 1D).

After 8 weeks of culture on MS medium supplemented with the three plant growth regulators, shoot induction ranged from 51.67% to 100% (Table 1). BA induced shoot formation at 76.67–80.00% (Figure 2A–C), while mT promoted shoot induction at 51.67–65.00% (Figure 2D–F). In contrast, TDZ induced 100% shoot formation across all tested concentrations (Figure 2G–I). The highest number and average shoot length were observed at 3.0 mg L^{-1} TDZ, with an average of 36.80 shoots per seed and a shoot length of 4.24 cm per seed. These results indicate that TDZ is highly effective for mass shoot induction in *S. guianensis* CIAT 184 under the tested *in vitro* conditions.

Root induction from shoots

Shoots derived from seedling cultures were excised and transferred to MS medium supplemented with 30 g L^{-1} sucrose and 2.6 g L^{-1} gellan gum, adjusted to pH 5.8. The medium was enriched with auxins, IAA, IBA, and NAA, at concentrations of 0.1, 0.3, and 0.5 mg L^{-1} to induce root formation. After 8 weeks of culture, all tested auxin treatments successfully induced root formation (Table 2). Among them, MS medium containing 0.3 mg L^{-1} IAA promoted the development of long roots with a white to light-brown coloration (Figure 3A-C). This treatment achieved 40% root induction, with an average of 6.5 roots per shoot and an average root length of 1.18 cm per shoot. MS medium supplemented with 0.5 mg L^{-1} IBA induced long, white roots with 36.67% root induction. Seedlings developed an average of 5.5 roots per plant, with an average root length of 1.12 cm (Figure 3D-F). In contrast, MS medium containing 0.3 mg L^{-1} NAA promoted the formation of short, brown roots, achieving 33.33% root induction, with an average of 4.4 roots per shoot and an average root length of 0.89 cm (Figure 3G-I). Overall, root induction across treatments ranged from an average of 3.6 roots per shoot with a mean root length of 3.90 cm per shoot.

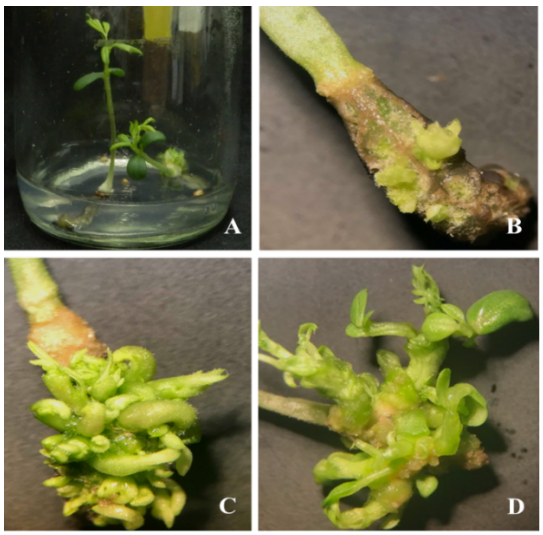

Figure 1. *In vitro* seed germination and shoot development of *S. guianensis* CIAT 184 on MS medium supplemented with 3 mg L^{-1} TDZ: (A) early germination for 5–7 days; (B) callus formation at the radicle; (C) shoot emergence from callus for 2 weeks; and (D) multiple shoots regenerated from callus for 8 weeks

Table 1. Shoot induction from seeds of *S. guianensis* CIAT 184 cultured on MS medium supplemented with different concentrations of the plant growth regulators BA, *m*T, and TDZ for 8 weeks

PGR types	Conc. (mg L^{-1})	No. of seedlings	No. of shoots	Shoot induction (%)	No. of shoots per seed	Shoot length per seed (cm)
BA	0.5	60	46	76.67	$3.00^{f}\pm0.31$	$2.71^{c}\pm0.30$
	1	60	47	78.33	$2.60^{f}\pm0.24$	$4.17^{a}\pm0.10$
	3	60	48	80.00	$7.20^{e}\pm0.20$	$4.16^{a}\pm0.09$
*m*T	0.5	60	31	51.67	$10.60^{d}\pm0.40$	$3.84^{ab}\pm0.21$
	1	60	38	63.33	$17.80^{c}\pm1.24$	$3.38^{b}\pm0.13$
	3	60	39	65.00	$23.40^{b}\pm3.20$	$4.24^{a}\pm0.06$
TDZ	0.5	60	60	100.00	$25.80^{b}\pm3.11$	$2.73^{c}\pm0.15$
	1	60	60	100.00	$34.80^{a}\pm1.87$	$3.86^{ab}\pm0.10$
	3	60	60	100.00	$36.80^{a}\pm0.83$	$4.24^{a}\pm0.11$

Note: PGR type = type of plant growth regulator, Mean ± SE calculated from five replicates According to Duncan's multiple range test, means within the same column followed by the same letter are not significantly different at $P \leq 0.05$.

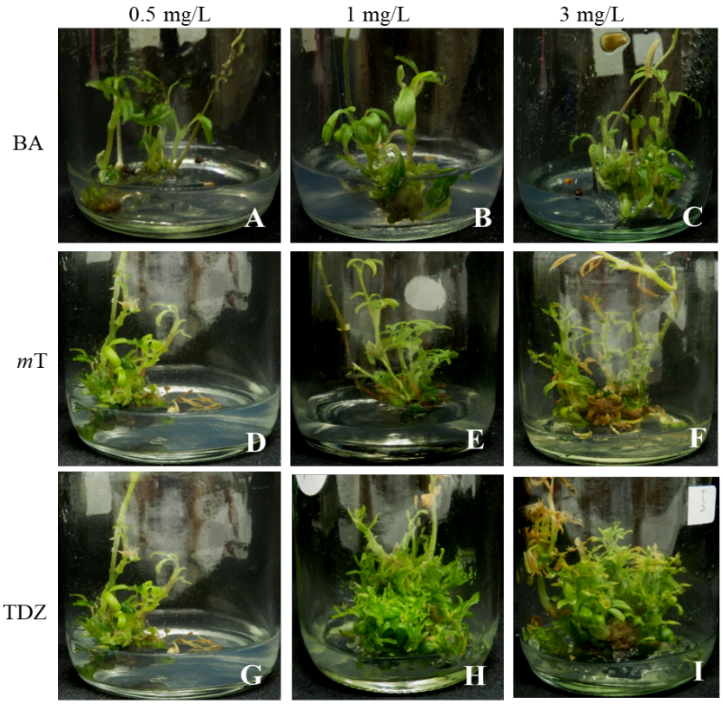

Figure 2. In vitro culture of *S. guianensis* CIAT 184 seeds on MS medium supplemented with different cytokinins for 8 weeks: (A–C) BA at 0.5, 1.0, and 3.0 mg L^{-1}; (D–F) mT at 0.5, 1.0, and 3.0 mg L^{-1}; (G–I) TDZ at 0.5, 1.0, and 3.0 mg L^{-1}

Table 2. Root induction from seedlings of *S. guianensis* CIAT 184 cultured on MS medium supplemented with different concentrations of IAA, IBA, and NAA for 8 weeks

PGR types	Conc. (mg·L^{-1})	No. of seedlings	No. of roots	Root induction (%)	No. of roots/ shoot	Root length/ shoot (cm)
IAA	0.1	30	4	13.33	$0.60^{e}\pm0.84$	$0.47^{c}\pm0.20$
	0.3	30	12	40.00	$6.50^{a}\pm6.38$	$1.18^{ab}\pm0.21$
	0.5	30	12	40.00	$2.80^{bcde}\pm2.20$	$0.88^{bc}\pm0.05$
IBA	0.1	30	11	36.67	$4.80^{abc}\pm0.38$	$1.12^{ab}\pm0.14$
	0.3	30	13	43.33	$3.70^{bcd}\pm0.59$	$1.33^{ab}\pm0.21$
	0.5	30	13	36.67	$5.50^{ab}\pm0.70$	$1.12^{ab}\pm0.12$
NAA	0.1	30	14	46.67	$2.70^{cde}\pm0.57$	$1.54^{a}\pm0.32$
	0.3	30	10	33.33	$4.40^{abcd}\pm0.45$	$0.89^{bc}\pm0.09$
	0.5	30	13	43.33	$4.00^{abcd}\pm0.81$	$1.10^{ab}\pm0.05$

Note: PGR type = type of plant growth regulator, Mean ± SE calculated from five replicates According to Duncan's multiple range test, means within the same column followed by the same letter are not significantly different at $P \leq 0.05$.

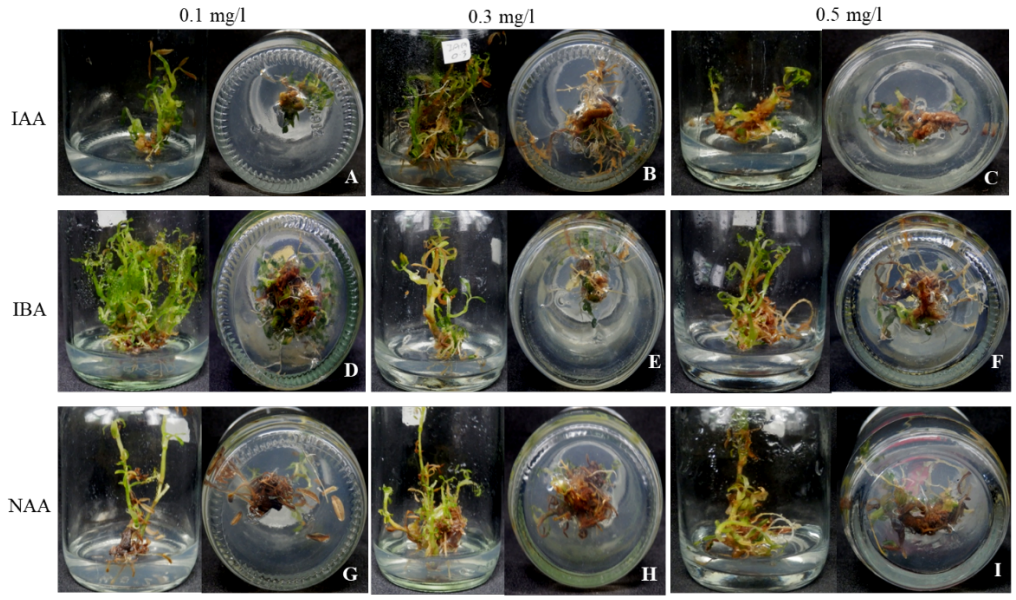

Figure 3. *In vitro* root development of *S. guianensis* CIAT 184 cultured on MS medium supplemented with various auxins for 8 weeks: (A-C) IAA at 0.1, 0.3, and 0.5 mg L^{-1}; (D-F) IBA at 0.1, 0.3, and 0.5 mg L^{-1}; (G-I) NAA at 0.1, 0.3, and 0.5 mg L^{-1}

Effect of gamma irradiation dose on seedlings

Mature seeds of *S. guianensis* CIAT 184 were exposed to acute gamma irradiation at doses of 0, 5, 10, 15, 20, 30, and 50 krad. Gamma irradiation exerted a clear dose-dependent effect, as seeds cultured on MS medium without growth regulators for 30 days showed markedly reduced germination, survival, and seedling growth at doses above 15-20 krad. For 30 days, the gamma doses causing 50% growth reduction (GR_{50}, 30) and 50% lethal dose (LD_{50}, 30) were determined to be 44.38 and 38.06 krad, respectively (Table 3, Figure 4A). Germination and survival remained at 100 % up to 10 krad and declined to 46.67 % and 32.14 %, respectively, at 50 krad. Shoot and root induction also decreased progressively with increasing dose, to 46.42% and 42.85%, respectively, at 50 krad. (Figure 4B). These results indicate that moderate gamma exposure (around 15-20 krad) promotes shoot elongation, whereas higher doses inhibit both germination and subsequent seedling development.

Table 3. Effects of gamma-ray dose on seed survival, germination, shoot and root formation, and shoot/root lengths of *S. guianensis* CIAT 184 after 30 days on MS medium without growth regulators

Dose (krad)	Germination (%)	Survival (%)	Shoot induction (%)	Shoot length (cm)	Root induction (%)	Root length (cm)
0	100.00	100.00	100.00	11.32^{b}±0.17	100.00	11.09^{a}±0.12
5	100.00	100.00	90.00	11.15^{b}±0.14	100.00	10.81^{ab}±0.07
10	100.00	100.00	83.33	11.10^{b}±0.19	100.00	10.54^{bc}±0.14
15	95.00	94.73	82.45	11.04^{bc}±0.11	96.49	10.53^{bc}±0.25
20	95.00	82.45	77.19	12.67^{a}±0.22	96.49	10.40^{bc}±0.05
30	71.67	62.79	69.76	10.50^{d}±0.14	58.13	10.27^{c}±0.07
50	46.67	32.14	46.42	6.29^{e}±0.26	42.85	8.46^{d}±0.18

Note: Number of seedlings = 60, Mean ± SE calculated from five replicates
According to Duncan's multiple range test, means within the same column followed by the same letter are not significantly different at $P \leq 0.05$.

Gamma irradiation also affected seedling performance of *S. guianensis* CIAT 184 cultured on MS medium supplemented with 3 mg L^{-1} TDZ for 60 days. Still, the adverse effects were less pronounced than on medium without growth regulators (Table 4, Figure 4C). Germination and survival remained at or near 100 % up to 20 krad and remained above 88.33 % at 50 krad, while shoot induction remained ≥ 95 % across all treatments, except at 50 krad. Shoot length peaked at 4.49 cm at 15 krad and gradually declined to 2.75 cm at 50 krad, and root length decreased slightly from 1.78 cm in the control to 1.28 cm at 50 krad. Compared with seedlings cultured without TDZ, those grown on TDZ medium exhibited much higher survival and shoot induction at high irradiation levels, indicating that TDZ mitigates the growth-suppressing effects of gamma rays and supports shoot regeneration under irradiation stress.

Table 4. Effects of gamma-ray dose on seed survival, germination, shoot and root formation, and shoot/root lengths of *S. guianensis* CIAT 184 after 60 days on MS medium with 3 mg L^{-1} TDZ

Dose (krad)	Germination (%)	Survival (%)	Shoot induction (%)	Shoot length (cm)	Root induction (%)	Root length (cm)
TDZ	100.00	100.00	100.00	3.66^{b}±0.75	100.00	1.78^{a}±0.05
5	100.00	100.00	100.00	3.74^{b}±0.92	100.00	1.74^{a}±0.41
10	100.00	100.00	100.00	3.71^{b}±1.01	100.00	1.72^{a}±1.40
15	100.00	100.00	100.00	4.49^{a}±1.42	100.00	1.63^{ab}±0.50
20	95.00	100.00	95.00	3.58^{ab}±0.81	95.00	1.47^{bc}±1.05
30	95.00	100.00	95.00	3.28^{c}±1.64	95.00	1.35^{c}±0.45
50	88.33	92.45	88.33	2.75^{d}±1.23	88.33	1.28^{c}±0.29

Note: Number of seedlings = 60, Mean ± SE calculated from five replicates
According to Duncan's multiple range test, means within the same column followed by the same letter are not significantly different at $P \leq 0.05$.

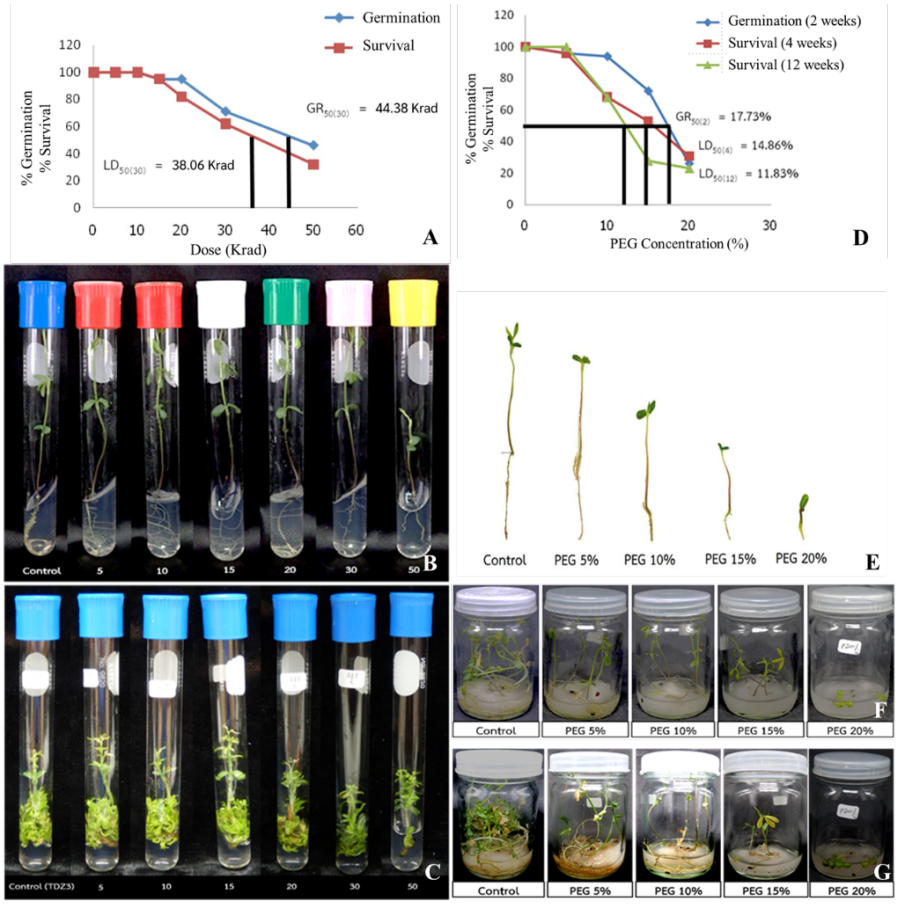

Figure 4. (A) Relationship between gamma-ray dose and seed germination and survival of *S. guianensis* CIAT 184 for 30 days on MS medium without growth regulators; (B) Seedling development from gamma-irradiated seeds on MS medium without growth regulators for 30 days; (C) Seedling development from gamma-irradiated seeds on MS medium supplemented with 3 mg L^{-1} TDZ for 60 days; (D) Relationship between PEG concentration and seed germination and survival for 30 days on MS medium without growth regulators; (E–G) Seedling development on MS medium supplemented with different PEG concentrations for 2 weeks (E), 4 weeks (F), and 12 weeks (G)

Seed drought tolerance

PEG-induced drought stress markedly reduced germination, survival, and seedling growth of *S. guianensis* CIAT 184 during 12 weeks on MS medium. At 0 % PEG, germination reached 100 %, but survival declined from 72 % at 4 weeks to 26 % at 12 weeks. Shoot and root lengths decreased over time, from 6.81 cm and 13.93 cm at 2 weeks to 0.94 cm and 0.63 cm at 12 weeks,

respectively. Increasing PEG concentrations further suppressed growth. At 5 % PEG, survival remained 100 % after 12 weeks, but shoot and root lengths decreased to 9.77 cm and 8.33 cm. At 10–20 % PEG, survival dropped sharply (68–23 %), accompanied by marked reductions in shoot (6.89–2.44 cm) and root lengths (2.74–0.66 cm). Germination decreased progressively with increasing PEG, with a GR_{50} of 17.73 % PEG. Seedling sensitivity was age-dependent: LD_{50} was 14.86 % PEG for 4-weeks-old seedlings and 11.83 % PEG for 12-weeks-old seedlings (Figure 4D), seedling development on MS medium with different PEG concentrations at 2, 4, and 12 weeks (Figure 4E-G), indicating that older seedlings are more susceptible to PEG-induced stress than younger ones, and that germination is slightly more tolerant than seedling survival.

Discussion

Effect of growth regulators on shoot induction, the present study clearly demonstrates that TDZ is highly effective in stimulating prolific shoot formation in *S. guianensis* CIAT 184 under *in vitro* conditions. This observation is in line with the findings of Rizvi and Singh (2000), who reported that TDZ efficiently triggers both organogenesis and somatic embryogenesis in chickpea (*Cicer arietinum*). In our experiment, TDZ-treated seedlings produced compact, robust shoots with a characteristically short stature. Such morphological traits are consistent with the well-established role of TDZ in promoting callus proliferation, somatic embryogenesis, and the generation of multiple shoots at relatively low concentrations, often surpassing the performance of other commonly used cytokinins. Comparable results were described by Basalma *et al*. (2008), who recorded the highest shoot induction rates in *Astragalus cicer* L. on MS medium containing TDZ. Furthermore, Mok *et al*. (2000) reported that TDZ exhibits strong cytokinin-like activity and can effectively replace conventional cytokinins such as BA or zeatin in plant tissue culture systems, supporting its broad applicability in shoot induction protocols.

The present study demonstrated that root induction from *S. guianensis* CIAT 184 shoots is strongly influenced by the type and concentration of auxins used. Among the tested treatments, MS medium supplemented with 0.3 mg L^{-1} IAA resulted in the highest root induction rate, producing the greatest number of roots per shoot (6.5) and the longest average root length (1.18 cm), with roots exhibiting a healthy white to light-brown coloration. IBA at 0.5 mg L^{-1} also promoted the formation of long, white roots, albeit with slightly lower root number and length, while NAA at 0.3 mg L^{-1} induced shorter and browner roots, suggesting that root quality and growth vigor are auxin-dependent. These findings align with previous reports that IAA and IBA effectively stimulate in vitro root formation in leguminous and woody plants. In contrast, NAA may

induce callus or shorter roots depending on species and concentration (Barik *et al*., 2006). The observed differences in root morphology may also be linked to auxin-mediated ethylene production, which can modulate ABA biosynthesis and inhibit growth at higher concentrations, as described by Hansen and Grossmann (2000). Overall, IAA at 0.3 mg L^{-1} appears optimal for promoting vigorous root development suitable for subsequent acclimatization and plantlet establishment.

Exposure of *S. guianensis* CIAT 184 seeds to gamma irradiation exhibited a clear dose-dependent effect on germination, seedling survival, and growth. Germination and survival remained unaffected at low doses (≤10 krad), whereas higher doses (15–20 krad) caused significant reductions in both parameters, with the lowest values observed at 50 krad. Shoot and root induction, as well as elongation, also declined progressively with increasing gamma dose, indicating that high irradiation levels inhibit meristem activity and overall seedling vigor. Interestingly, moderate doses of 15–20 krad appeared to slightly promote shoot elongation, which may be related to sub-lethal stress stimulating growth hormone, as reported in other leguminous species (Beyaz *et al*., 2016). The calculated GR_{50} (44.38 krad) and LD_{50} (38.06 krad) further quantify the tolerance threshold of Stylo seeds under acute gamma exposure. These findings are consistent with previous studies showing that low to moderate gamma doses can enhance germination and early seedling growth, whereas higher doses inhibit development due to DNA damage and oxidative stress (Ngoenngam *et al*., 2019; Abdel-Hady *et al*., 2008). Overall, the results highlight the importance of optimizing gamma irradiation doses to achieve a balance between mutation induction and seedling survival for in vitro breeding programs.

The present study demonstrated that TDZ supplementation in MS medium mitigates the adverse effects of gamma irradiation on *S. guianensis* CIAT 184 seedlings. Seeds cultured on MS medium containing 3 mg L^{-1} TDZ for 60 days maintained high germination and survival rates even at 50 krad (88.33 % and 92.45 %, respectively), while shoot induction remained ≥88 % across all treatments. Shoot length peaked at 15 krad (4.49 cm) and gradually declined at higher doses, whereas root length decreased slightly from 1.78 cm in the control to 1.28 cm at 50 krad. Compared with seedlings grown on MS medium without growth regulators, those cultured with TDZ showed markedly higher survival and shoot induction at elevated irradiation levels, indicating that TDZ supports shoot regeneration and reduces growth suppression under gamma stress. These observations are consistent with previous reports that TDZ promotes adventitious shoot regeneration and enhances tissue tolerance to abiotic stress in in vitro cultures. The protective effect of TDZ may be attributed to its cytokinin-like activity, which stimulates cell division and organogenesis, thereby counteracting irradiation-induced growth inhibition.

The results of this study demonstrate that PEG-induced drought stress significantly affects germination, seedling survival, and growth of *S. guianensis* CIAT 184 in a dose- and age-dependent manner. Germination remained relatively tolerant to low PEG concentrations, whereas seedling survival and growth were progressively inhibited as PEG concentration increased, with the most pronounced effects observed at 20 % PEG. Older seedlings (12 weeks) were more sensitive to drought stress than younger seedlings (4 weeks), as reflected by lower LD_{50} values (11.83 % vs. 14.86 %). Shoot and root growth declined markedly with both increasing PEG concentration and culture duration, indicating that prolonged exposure exacerbates stress effects. These findings are consistent with previous reports on millet species, where PEG treatment reduced germination, seedling survival, and early growth, with older or more developed seedlings showing greater sensitivity (Bheemesh *et al*., 2018; Daouda Ousmane and Mouhamadou Mounkaila, 2014). The observed differential tolerance between germination and seedling survival suggests that early germination mechanisms are more resilient to osmotic stress, whereas subsequent seedling development is highly susceptible to water deficit. Overall, PEG-mediated drought stress provides an effective in vitro model for assessing the drought tolerance of S. guianensis CIAT 184 and identifying resilient seedlings for further propagation.

The study demonstrated that TDZ efficiently promotes shoot formation and IAA (0.3 mg L^{-1}) optimizes root induction in *S. guianensis* CIAT 184. Gamma irradiation showed dose-dependent effects, while TDZ mitigated its adverse impacts, and PEG-induced drought stress highlighted greater sensitivity in older seedlings. These results guided *in vitro* propagation, mutation breeding, and early selection of drought-tolerant seedlings. Future work should validate these findings under ex vitro or field conditions, assessing plantlet establishment, stress tolerance, and productivity to support breeding and cultivation strategies.

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Conflicts of interest

The authors declare no conflict of interest.

References

Abdel-Hady, M. S., Okasha, E. M., Soliman, S. S. A. and Talaat, M. (2008). Effect of gamma radiation and gibberellic acid on germination and alkaloid production in *Atropa belladonna* L. Australian Journal of Basic and Applied Sciences, 2:401-405.

Barik, D., Mohapatra, U. and Chand, P. (2006). Direct shoot regeneration from epicotyl explants of grasspea (*Lathyrus sativus*). Australian Journal of Botany, 54:505-508.

Basalma, D., Uranbey, S., Gürlek, D. and Özcan, S. (2008). TDZ-induced plant regeneration in *Astragalus cicer* L. African Journal of Biotechnology, 7:955-959.

Bheemesh, S. J., Subba Rao, M., Reddi Sekhar, M. and Latha, P. (2018). Effect of PEG-induced drought stress on germination and early seedling growth of foxtail millet varieties. International Journal of Current Microbiology and Applied Sciences, 6:2674-2681.

Beyaz, R., Kahramanogullari, C. T., Yildiz, C., Darcin, E. S. and Yildiz, M. (2016). The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under in vitro conditions. Journal of Environmental Radioactivity, 162-163:129-133.

Daouda Ousmane, S. and Mouhamadou Mounkaila, B. (2014). Effect of polyethylene glycol (PEG) 6000 on germination and seedling growth of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and LD_{50} for in vitro screening for drought tolerance. African Journal of Biotechnology, 13:3742-3747.

Hansen, H. and Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. Plant Physiology, 124:1437-1448.

Kiyothong, K., Satjipanon, C. and Pholsen, P. (2002). Effect of cutting height and time on seed yield and seed quality of *Stylosanthes guianensis* CIAT 184. Songklanakarin Journal of Science Technology, 24:587-593.

Mok, M. C., Martin, R. C. and Mok, D. W. S. (2000). Cytokinins: Biosynthesis, metabolism and perception. In Vitro Cellular & Developmental Biology – Plant, 36:102-107.

Murashige, T. and Skoog, F. (1962). A revised medium for growth and bioassays with tobacco tissue. Physiology Plant, 15:473-497.

Ngoenngam, L., Pongtongkam, P., Arananant, J., Poeaim, S. and Poeaim, A. (2019). In vitro effect of gamma irradiation and plant growth regulators (PGRs) for induction and development of *Stylosanthes hamata* cv. Verano. International Journal of Agricultural Technology, 15:63-74.

Pandit, R., Bhusal, B., Regmi, R., Neupane, P., Bhattarai, K., Maharjan, B., Acharya, S., Bigyan K. C. and Poudel, M. R. (2021). Mutation breeding for crop improvement: A review. Reviews in Food and Agriculture, 2:31-35.

Rizvi, S. M. H. and Singh, R. P. (2000). In vitro plant regeneration from immature leaflet-derived callus cultures of *Cicer arietinum* L. via organogenesis. Plant Cell Biotechnology and Molecular Biology, 1:109-114.

Rupitak, Q. and Srisaikham, S. (2021). Evaluation of yield production and chemical composition of three types of forage legumes at different cutting intervals and cutting times to assess their benefits as ruminant animal feed. Chiang Mai University Journal of Natural Sciences, 20:e2021046.

Schultze-Kraft, R., Hubiao, Y., Jun, T. and Guodao, L. (2023). *Stylosanthes guianensis* CIAT 184 – review of a tropical forage legume. Tropical Grasslands-Forrajes Tropicales, 11:95-120.

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