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Research Article
Induction of Buds by Cold and Gibberellic Acid Pretreatment of Potato Tubers (*Solanum tuberosum* L.) for *in vitro* Plant Regeneration

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Abstract

Background and Objective: Potato (*Solanum tuberosum* L.) is a globally important food crop, yet its cultivation in tropical regions is limited by the absence of an efficient local seed production system. This study aimed to develop an integrated and reproducible protocol for *in vitro* regeneration of potato adapted to tropical environments. **Materials and Methods:** Tubers of two cultivars, Roseval and Charlotte, were subjected to four preconditioning treatments combining Gibberellic Acid (GA_3) and cold exposure to enhance bud induction before culture initiation. Excised buds were disinfected with Sodium Hypochlorite (NaOCl) or Calcium Hypochlorite [$Ca(ClO)_2$] at concentrations of 0.5, 1.0, and 2.0% active chlorine for varying exposure times. Regenerated plantlets were multiplied through successive subcultures on Murashige and Skoog (MS) medium supplemented with Morel and Wetmore vitamins and subsequently acclimatized on different substrates. Data were analyzed in R (v4.3.3) with arcsine-transformed percentages, ANOVA for cultivar, pretreatment, and disinfection effects, and Tukey's HSD test for significant differences ($p<0.05$). **Results:** The combined GA_3+cold pretreatment significantly increased bud formation (20.3 buds per tuber in Roseval and 12.4 in Charlotte), confirming a synergistic effect on dormancy release and sprout development. Optimal disinfection was achieved using 1% active chlorine for 8 min, producing more than 86% healthy plantlets for both disinfectants. During acclimatization, sand and forest soil provided the highest survival rates (96.7% in Roseval and over 80% in Charlotte). **Conclusion:** This integrated approach harmonized pretreatment, disinfection, and acclimatization steps, enabling the efficient regeneration of vigorous and disease-free potato plantlets. The optimized protocol establishes a technical foundation for local seed potato production in West Africa, thereby contributing to regional food security and sustainable crop propagation.

Key words: *Solanum tuberosum*, gibberellic acid, cold pretreatment, *in vitro* culture, disinfection, acclimatization

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The potato (*Solanum tuberosum* L.) ranks among the world's most important food crops after rice and wheat, serving as a major source of carbohydrates, vitamins, and dietary fiber[1]. Despite its global significance, potato cultivation remains underdeveloped in many tropical regions, particularly in West Africa, where local seed production systems are either inefficient or absent. As a result, the regional potato supply relies heavily on costly imports, increasing production expenses and disease risks associated with the repeated use of uncertified seed tubers[2]. Developing locally adapted *in vitro* propagation systems is therefore essential to ensure the sustainable production of high-quality, disease-free planting material.

Micropropagation has become a reliable technique for producing genetically uniform and pathogen-free potato plantlets, allowing rapid multiplication and consistent plant quality[3,4]. However, the success of micropropagation largely depends on the physiological status of the explant, its response to growth regulators, and the preconditioning treatments applied before culture initiation[5,6]. In potatoes, dormancy of freshly harvested tubers frequently delays sprout emergence, restricting their immediate use as explant material. Dormancy is a complex physiological state regulated by endogenous hormonal balance and environmental cues such as temperature, light, and storage duration[7].

Gibberellic Acid (GA_3) is widely used to break dormancy and stimulate bud sprouting by promoting cell elongation and activating metabolic pathways involved in carbohydrate mobilization[8,9]. Similarly, exposure to cold storage has been shown to accelerate dormancy release and improve sprout uniformity by altering hormonal ratios and reducing abscisic acid accumulation[10,11]. However, few studies have explored the combined effect of GA_3 and low temperature on bud induction and *in vitro* responsiveness, especially under tropical conditions, where potato physiology may differ substantially from that of temperate varieties. Understanding this interaction could provide valuable insights for optimizing explant performance in biotechnological applications.

Another crucial factor influencing *in vitro* success is surface sterilization. Contamination by endogenous or exogenous microorganisms often compromises culture establishment, particularly when field-grown material is used. Sodium Hypochlorite (NaOCl) and Calcium Hypochlorite [$Ca(ClO)_2$] are commonly employed disinfectants due to their broad antimicrobial activity and relatively low phytotoxicity[12,13]. However, their optimal concentrations and exposure times must be precisely calibrated to achieve a balance between sterilization efficiency and explant viability.

In Côte d'Ivoire and across West Africa, research on potato tissue culture remains limited compared with that in temperate regions. To our knowledge, this study is the first to integrate hormonal and cold preconditioning, optimized disinfection, and *ex vitro* acclimatization into a single reproducible protocol tailored for tropical potato production systems. We hypothesized that combining GA_3 pretreatment with cold exposure would enhance the physiological competence of tuber buds, resulting in higher regeneration efficiency and acclimatization success. Therefore, the objectives of this study were to (1) Evaluate the effects of GA_3 and cold pretreatments on bud induction, (2) Determine optimal disinfection parameters for explant establishment, and (3) Assess acclimatization performance on different substrates. This integrated approach is expected to form the basis for sustainable seed potato production and crop improvement initiatives in Sub-Saharan Africa.

MATERIALS AND METHODS

Study area: The study was conducted from February, 2023 to January, 2024 at the Laboratory of *in vitro* culture of WASCAL (West African Science Service Centre on Climate Change and Adapted Land Use) in Abidjan (Côte d'Ivoire).

Plant material: Tubers of two Solanum tuberosum cultivars, Roseval and Charlotte, were used in this study. The tubers, previously treated with anti-sprouting agents, were imported from SARL LESCIEUX (Juniville, France) and purchased from Hyper U supermarkets in Abidjan, Côte d'Ivoire. Uniformly sized tubers (35-55 mm in diameter), free from visible mechanical damage or disease symptoms, were carefully selected (Fig. 1). All tubers were washed three times with a mild detergent, thoroughly rinsed with tap water, and air-dried before pretreatment.

Methods

preconditioning and *in vivo* bud induction: Preconditioning treatments were designed to promote bud induction and improve explant quality before *in vitro* culture. Four treatments were tested:

- **T0 (Control):** Tubers incubated in complete darkness at room temperature, without pretreatment
- **T1:** Tubers soaked in a 2 mg/L GA_3 solution for 1 hr, drained, and incubated in darkness
- **T2:** Tubers stored at 5°C for 7 days, then transferred to room temperature in darkness
- **T3:** Tubers soaked in 2 mg/L GA_3 for 1 hr, stored at 5°C for 7 days, and subsequently maintained in darkness

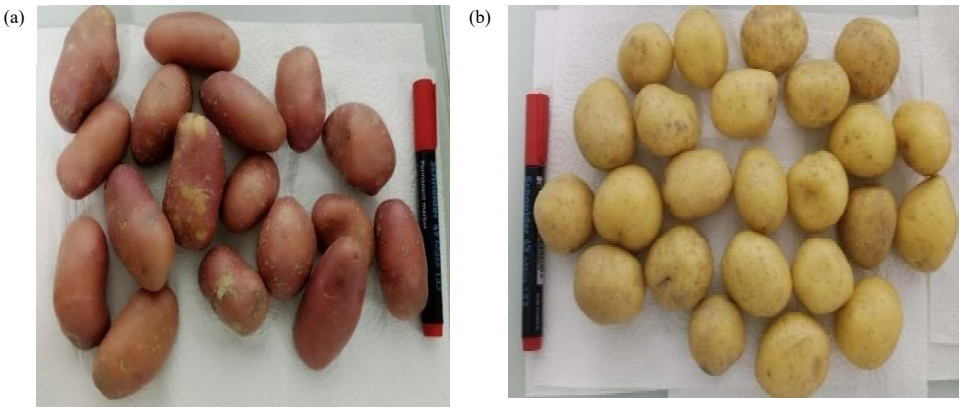

Fig. 1(a-b): Potato tubers of the tested cultivars: (a) Roseval and (b) Charlotte

After pretreatment, all tubers were placed in wooden crates and kept in total darkness for 3 days. They were then transferred to a growth chamber under a 16 hrs light/8 hrs dark photoperiod at 25±2°C and 80% relative humidity for 14 days. Each treatment included 90 tubers per replicate, with three independent repetitions. The recorded parameters per tuber included the number of buds, bud height (cm), and the number of nodes per shoot.

Explant disinfection and *in vitro* bud induction: Buds approximately 14 days old were excised from preconditioned tubers and used as explants. Each disinfection treatment was applied to 60 explants per replicate, with three replicates per treatment.

Explants were first washed under running tap water for 10 min with a few drops of liquid soap, then rinsed and transferred to a laminar airflow cabinet. They were immersed in 70% (v/v) ethanol[3] for 30 sec, followed by disinfection with either Sodium Hypochlorite (NaOCl) or Calcium Hypochlorite [$Ca(ClO)_2$] at concentrations of 0.5%, 1.0%, or 2.0% active chlorine for 10 min[12,13]. The optimal concentration (1%) was then evaluated at exposure times of 5, 8, and 15 min.

After disinfection, explants were rinsed five times with sterile distilled water and inoculated onto Murashige and Skoog (MS) medium[14], supplemented with 1 mL/L Morel and Wetmore vitamins[15], 30 g/L sucrose, and 2.5 g/L gellan gum (Gelrite) for *in vitro* buds' induction. Owing to its mineral richness, this medium is well suited for the *in vitro* culture of potato, and the species' strong apical dominance can be effectively overcome by culturing explants (buds) on a hormone-free medium[6]. The medium pH was adjusted to 5.8 before autoclaving at 121°C for 20 min.

Cultures were incubated at 25±2°C under a 16 hrs photoperiod (100 µmol·m^{-2}/sec) provided by cool white fluorescent lamps and maintained at 80% relative humidity.

Disinfection efficiency was evaluated 21 days after inoculation based on contamination rate, necrosis rate, and percentage of healthy explants. Growth parameters including plantlet height, number of shoots, rooting rate, and number of nodes, were measured 30 days after culture initiation.

Acclimatization: Vitroplants displaying normal morphology and a well-developed root system (at least three roots longer than 2 cm) were selected for acclimatization. These experiments were conducted using potting soil, forest soil and washed sea sand, substrates widely used for *in vitro* plants acclimatization[16,17].

The potting soil contained approximately 86% organic matter, had a pH of 6.5, moderate electrical conductivity (4.5 mS/m), and a 60% water-holding capacity, and was fertilized with NPK(6-5-7). Forest soil was collected from the surface humus layer, while sea sand was thoroughly washed to remove salts and residues. All substrates were sieved (2 mm mesh), moistened, and sterilized by autoclaving at 121°C for 20 min before use. Seven substrate formulations were prepared: T (100% potting soil), Tf (100% forest soil), S (100% sand), T-Tf (50:50 potting soil+forest soil, v/v), T-S (50:50 potting soil+sand, v/v), Tf-S (50:50 forest soil+sand, v/v), and T-Tf-S (50:25:25 potting soil+forest soil+sand, v/v/v).

After four subculture cycles, plantlets were gently removed from the culture medium, rinsed with sterile distilled water to eliminate agar residues, and transferred into 180 mL plastic pots containing the prepared substrates. The pots were covered with transparent polyethylene lids to maintain high humidity (80-90%) while allowing light penetration.

For each substrate, 60 plantlets per cultivar were acclimatized, and the experiment was repeated three times. Acclimatization lasted 30 days in two stages: During the first 10 days, plantlets were maintained in a growth chamber at 25±2°C under a 16 hrs light photoperiod and 80% relative

humidity to facilitate a gradual transition from *in vitro* to *ex vitro* conditions. The covers were then progressively removed over six days to reduce humidity and promote hardening. Subsequently, the plants were transferred to a greenhouse for continued growth. The survival rate (%) was recorded 30 days after transfer.

Statistical analysis: All data were analyzed using R software (version 4.3.3). Analysis of Variance (ANOVA) was performed to evaluate the effects of cultivar, pretreatment, and disinfection protocol. When significant differences were detected ($p<0.05$), means were compared using Tukey's HSD test.

RESULTS

Effect of pre-treatments on *in vivo* bud induction: The number of buds produced per tuber varied significantly according to both the pretreatment and the cultivar ($p<0.001$). Overall, the cultivar Roseval exhibited a greater capacity for bud formation than Charlotte under all treatment conditions (Fig. 2a). The combined GA_3 and cold treatment (T3) produced the highest number of buds, averaging 20.3 buds per tuber in Roseval and 12.4 in Charlotte. No significant differences ($p>0.05$) were observed among treatments for the number of nodes or bud height Fig. 2(b-c). However, clear morphological differences were noted across treatments. Control tubers (T0) developed few, short buds, whereas GA_3 alone (T1) induced elongated and slender sprouts. Cold treatment (T2) promoted compact buds of moderate number, while the combined GA_3+cold pretreatment (T3) generated numerous, robust, and uniformly developed buds in both cultivars (Fig. 3).

Visual observations confirmed that GA_3 primarily enhanced elongation, whereas cold exposure favored compactness. Their combination yielded the most vigorous and physiologically active buds, particularly in Roseval, which showed a stronger response to GA_3 and temperature variation than Charlotte.

Effect of disinfectant concentration on bud disinfection: The efficacy of NaOCl and $Ca(ClO)_2$ in controlling contamination and necrosis was strongly dependent on concentration (Table 1). Increasing the concentration from 0.5% to 2% effectively reduced contamination but simultaneously

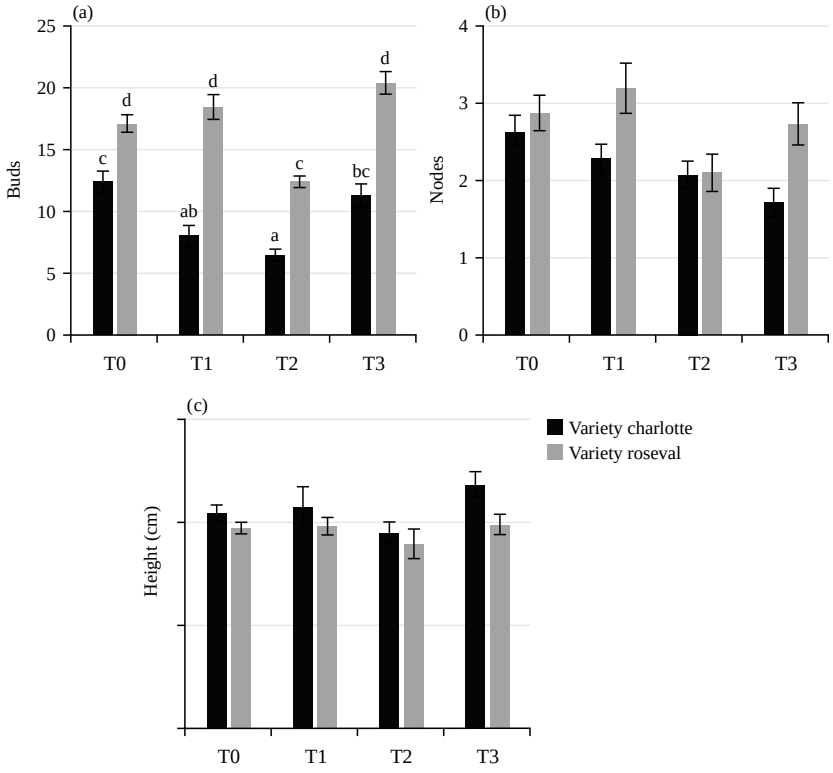

Fig. 2(a-c): Influence of pre-treatment on bud induction parameters in the potato varieties Roseval and Charlotte, (a) Number of buds ($p<0.001$), (b) Number of nodes ($p = 0.0801$) and (c) Bud height ($p = 0.634$)

T0: control, untreated tubers, T1: Soaking in a GA_3 solution (2 mg/L) for 1 hrs, T2: Storage at 5°C for 7 days, T3: Soaking in GA_3 (2 mg/L, 1 hrs) followed by storage at 5°C for 7 days. Bars sharing the same letter are not significantly different at the 5% level. Error bars represent the standard error (SE)

Fig. 3: Buds induced on potato tubers of the Charlotte (beige) and Roseval (burgundy) varieties
T0: control, tubers incubated in a culture room without treatment, T1: tubers soaked in a GA_3 solution (2 mg/L) for 1 hrs, T2: tubers stored at 5°C for 7 days only and T3: tubers soaked in a GA_3 solution (2 mg/L) for 1 hrs and then stored at 5°C for 7 days

Table 1: Effects of different concentrations of NaOCl and $Ca(ClO)_2$ on contamination, necrosis, and healthy plantlets

Disinfectant	Concentration (% active chlorine)	Contamination (%)	Necrosis (%)	Healthy plantlets (%)
NaOCl	0.5	72.22±2.78^c	0.00±00^a	27.78±2.78^a
	1	4.22±0.83^b	5.22±1.75^b	90.56±2.36^b
	2	0.00±00^a	71.11±2.61^c	28.89±2.61^a
	P	<0.001	<0.001	<0.001
$Ca(ClO)_2$	0.5	81.67±3.39^b	4.67±1.15^a	12.56±2.44^a
	1	8.11±1.42^a	7.22±1.84^a	84.67±2.42^b
	2	5.67±1.78^a	87.11±3.68^c	7.22±2.39^a
	P	<0.001	<0.001	<0.001

Values represent Mean±Standard Error (n = 3), Means followed by the same letter within a column are not significantly different according to Tukey's HSD test at $p \leq 0.05$

Table 2: Contamination rate, necrosis rate, and proportion of healthy vitroplants derived from buds subjected to different disinfection times with NaOCl and Ca(ClO) at 1% active chlorine

Disinfectants	Exposition time (min)	Contamination (%)	Necrosis (%)	Healthy plantlets (%)
NaOCl	5	30±2^b	5±0^a	70±1.15^b
	8	0.67±0.33^a	13±1.73^a	86.33±2.02^c
	15	0±0^a	50±5.77^b	50±5.77^a
	P	<0.001	<0.001	<0.001
$Ca(ClO)_2$	5	80±0^c	0±0^a	20±0^a
	8	13.67±3.33^b	0±0^a	83.33±3.33^c
	15	0±0^a	33.33±3.33^b	66.67±3.33^b
	P	0.001	<0.001	<0.001

Means followed by the same letter within the same column are statistically identical at the 5% significance level. Values are expressed as Mean±Standard Error. P: Probability, NaOCl: Sodium hypochlorite and $Ca(ClO)_2$: Calcium hypochlorite

increased tissue necrosis. At 0.5%, contamination levels remained high (72.2% for NaOCl and 81.7% for $Ca(ClO)_2$), whereas at 2%, necrosis rates increased sharply (71.1% and 87.1%, respectively). The intermediate concentration of 1% active chlorine provided the most effective balance between sterilization efficiency and explant survival, producing 90.6% healthy plantlets with NaOCl and 84.7% with $Ca(ClO)_2$.

Effect of exposure time on bud disinfection: Exposure time had a significant effect on contamination, necrosis, and explant survival for both disinfectants (Table 2). Short exposure (5 min) resulted in partial sterilization and moderate contamination (30% for NaOCl and 80% for $Ca(ClO)_2$), whereas prolonged exposure (15 min) caused severe necrosis and markedly reduced plantlet survival. An 8-minute exposure at

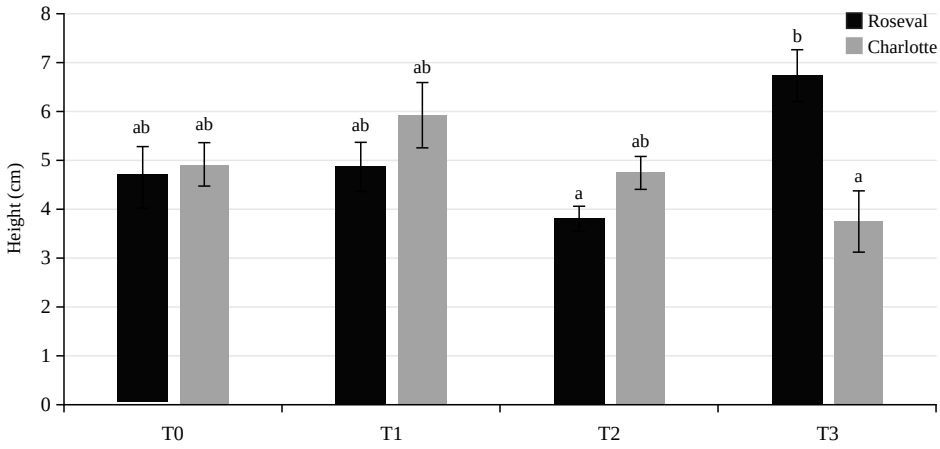

Fig. 4: Influence of the interaction between variety and preconditioning on *in vitro* plantlet shoot height
Bars followed by the same letter are not significantly different at the 5% level p<0.001

Table 3: Growth parameters of vitroplants developed after bud pre-treatment and disinfection

Varieties	Disinfection protocols	Pre-treatment	No. of nodes	No. of leafy shoots	Rooting rate
Roseval	NaOCl	T0	10.40±1.04^{a}	5.00±0.89^{a}	100
		T1	9.30±1.25^{ab}	2.70±0.62^{b}	100
		T2	4.10±0.43 ^{c}	1.40±0.16^{c}	100
		T3	6.60±0.86^{bc}	1.50±0.17^{c}	100
	Ca(ClO)	T0	4.96±1.17^{c}	2.50±0.56^{b}	100
		T1	10.70±1.27^{a}	2.80±0.42^{b}	100
		T2	8.50±1.42^{ab}	2±0.37^{b}	100
		T3	8.50±1.75^{ab}	1.50±0.34^{c}	100
Charlotte	NaOCl	T0	6.10±1.20^{b}	1.20±0.13^{bc}	100
		T1	7.50±0.93^{ab}	0.90±0.18^{c}	100
		T2	5.60±0.65^{b}	1±0.00^{bc}	100
		T3	4.90±0.75^{bc}	1.20±0.13^{bc}	100
	Ca(ClO)	T0	6.90±1.25^{b}	1±0.15^{bc}	100
		T1	6.10±1.22^{b}	0.80±0.13^{c}	100
		T2	6.20±0.61^{b}	1.10±0.10^{bc}	100
		T3	4.80±1.35^{bc}	1.10±0.23^{bc}	100
	P		<0.05	<0.05 -	

Means followed by the same letter within the same column are statistically identical at the 5% significance level. Values are expressed as Mean±Standard Error. P: probability, T0: control, tubers incubated in a culture room without treatment, T1: tubers soaked in a GA_3 solution (2 mg/L) for 1 hrs, T2: tubers stored at 5°C for 7 days, T3: tubers soaked in a GA_3 solution (2 mg/L) for 1 hrs and then stored at 5°C for 7 days

1% active chlorine achieved the most favorable results, providing nearly complete sterility and the highest proportion of healthy explants (86.3% for NaOCl and 83.3% for $Ca(ClO)_2$).

Interaction of variety, pretreatment and disinfection on *in vitro* growth: A significant three-way interaction among cultivar, pretreatment, and disinfection protocol was detected for both node number and leafy shoot production ($p<0.05$), whereas rooting percentage remained unaffected and reached 100% across all treatment combinations (Table 3). Node production exhibited marked variation depending on the specific treatment combination. In Roseval, the highest node number was recorded under GA pretreatment (T1) combined with Ca(ClO) disinfection (10.70 nodes per explant), closely followed by the untreated control (T0) disinfected with NaOCl (10.40 nodes). The lowest value was observed under cold storage alone (T2) combined with NaOCl (4.10 nodes).

In Charlotte, node production ranged from 4.80 to 7.50 nodes per explant, with the maximum obtained under GA_3 pretreatment combined with NaOCl disinfection (T1) and the minimum under T3 combined with $Ca(ClO)_2$. Leafy shoot production followed a similar interaction pattern. In Roseval, the highest value was observed in untreated tubers disinfected with NaOCl (5.00 shoots per explant), whereas other treatment combinations produced between 1.40 and 2.80 shoots. In contrast, Charlotte displayed lower and less variable shoot production, ranging from 0.80 to 1.20 shoots per explant across all combinations. These results indicate that

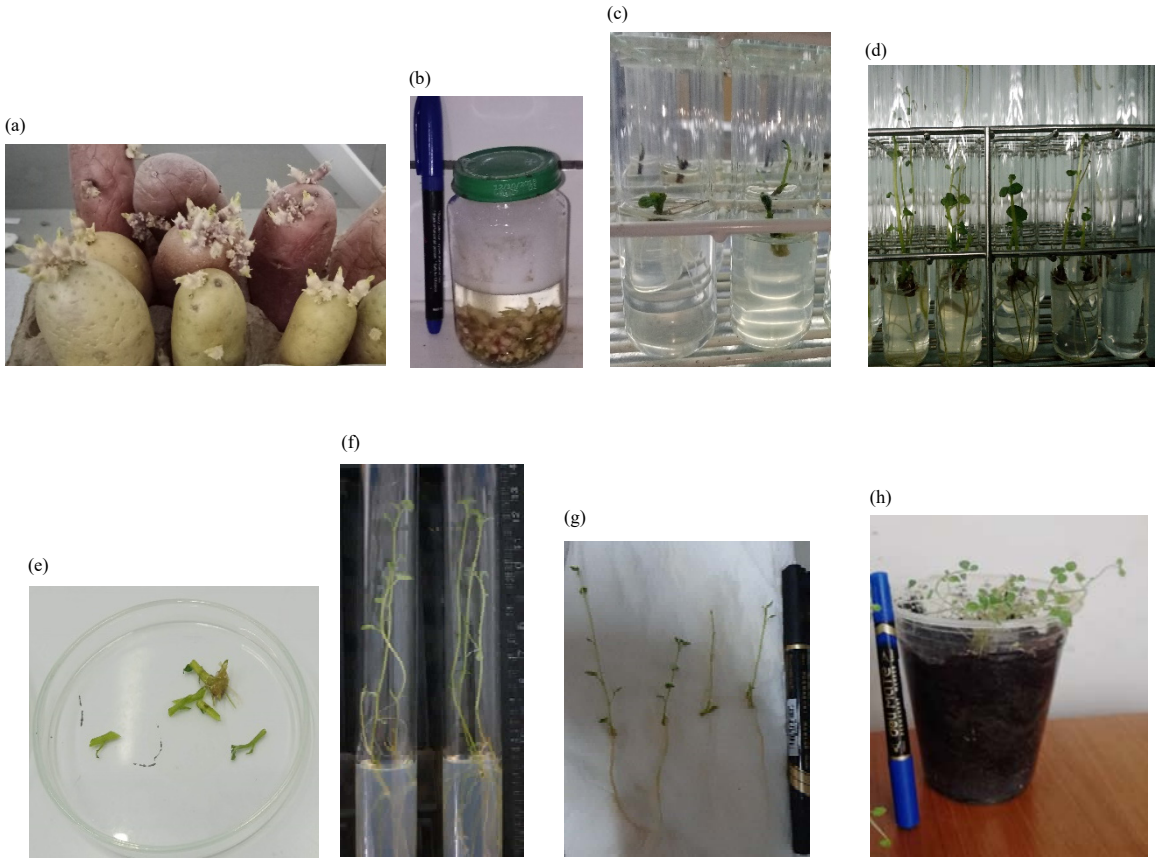

Fig. 5(a-h): Regeneration protocol of the two potato cultivars, (a) Buds induced after T3 preconditioning, (b) Disinfection of buds with 1% active chlorine, (c) Disinfected buds on medium, (d) Vitroplants obtained after disinfection, (e) Explants derived from the vitroplants after disinfection, (f) Vitroplants obtained after subculture, (g) Vitroplants removed from test tubes and washed prior to acclimatization and (h) Acclimatized vitroplants

Table 4: Survival rates of potato vitroplants of the roseval and charlotte varieties on different substrate types

Varieties	Substrates	Survival rates (%)
Roseval	T (100%)	46.67±16.67^{ab}
	S (100%)	96.67±3.33^{b}
	Tf (100%)	96.67±3.33^{b}
	T+S (1 :1)	90±10^{b}
	T+Tf (1 :1)	86.67±13.33^{b}
	Tf +S (1 :1)	90±10^{b}
	T+S+Tf (2 :1 :1)	93.33±6.67^{b}
Charlotte	T (100%)	46.67±6.67^{ab}
	S (100%)	80±15.27^{b}
	Tf (100%)	83.33±16.67^{b}
	T+S (1 :1)	6.67±3.33^{a}
	T+Tf (1 :1)	50±20^{ab}
	Tf+S (1 :1)	6.67±3.33^{a}
	T+S+Tf (2 :1 :1)	36.67±16.67^{ab}
	p	<0.01

T: Potting soil, Tf: Forest soil, and S: Sand used alone or in combination (1:1 or 2:1:1). Values are expressed as Mean±Standard Error. Different letters indicate significant differences according to Tukey's test ($p<0.05$) within the same column

morphogenic responses varied according to the combined effects of genotype, pretreatment, and disinfection protocol, while rooting capacity remained stable under all tested conditions.

Shoot elongation was mainly determined by the interaction between cultivar and pretreatment (Figure 4). The tallest plantlets (6.72 cm) developed from Roseval buds preconditioned with the combined GA_3+cold treatment (T3),

whereas the shortest shoots were observed in 'Charlotte' subjected to the same treatment and in Roseval exposed to cold alone (T2), with mean heights of 3.74 and 3.80 cm, respectively.

Acclimatization: Figure 5 illustrates the sequential stages of the regeneration protocol for both potato cultivars. Buds were first induced (a) and subsequently disinfected using 1% active chlorine (b). The resulting *in vitro* plantlets (c) were excised into uninodal segments (d) for subculturing. After three successive subculture cycles, the regenerated plantlets (e) were removed from the culture tubes, gently washed to eliminate agar residues, and transferred to the acclimatization phase (f). All these steps culminated in the production of fully acclimatized plants (g).

This final *ex vitro* transfer phase allowed the evaluation of plantlet adaptability to different substrate compositions. Acclimatization success was significantly affected by both substrate and cultivar (Table 4). The 'Roseval' cultivar achieved the highest survival rates on sand and forest soil (96.7%), followed by mixed substrates (T-S, Tf-S, and T-Tf-S), with survival exceeding 85%. In contrast, pure potting soil resulted in the lowest survival (46.7%).

Similarly, 'Charlotte' plantlets performed best on sand and forest soil (80-83%), whereas those grown on mixtures containing potting soil (T-S and Tf-S) showed poor adaptation, with survival dropping below 10%.

DISCUSSION

Control tubers produced few and short buds, reflecting incomplete natural dormancy release. In contrast, pretreatment with Gibberellic Acid (GA_3) markedly promoted sprouting and bud elongation. Gibberellins are well known to break dormancy and stimulate cell elongation by enhancing amylase synthesis and increasing cell wall extensibility[18,19]. Similar findings were reported by Muchiri *et al.*[9], who observed significant increases in sprout length and number following GA_3 application in potato seed tubers.

Cold preconditioning alone induced compact, short buds, consistent with studies showing that low temperature promotes dormancy release without promoting elongation[8,10]. The combined GA_3+cold treatment proved most effective, producing vigorous and well-developed buds. Transcriptomic analyses indicate that cold exposure upregulates *StGA3ox1* and *StGA20ox*, thereby enhancing endogenous GA biosynthesis and reducing abscisic acid accumulation factors that together synchronize sprouting[10,20].

The stronger response of the Roseval cultivar compared with Charlotte suggests genotype-dependent sensitivity to hormonal and thermal stimuli. Such variation has been linked to differential expression of genes involved in hormone metabolism (*StNCED1*, *DOG1*-like) and carbohydrate mobilization efficiency[21]. These observations corroborate previous reports showing that varietal responses to dormancy-breaking treatments depend on both the physiological state and genetic background of the tuber[9,18].

Both sodium hypochlorite (NaOCl) and calcium hypochlorite [$Ca(ClO)_2$] were effective disinfectants, although their performance depended strongly on concentration and exposure time. At 0.5%, contamination persisted, whereas 2% caused extensive tissue necrosis-results consistent with previous findings in other crops[22]. The intermediate treatment (1% active chlorine for 8 min) provided the best compromise between sterility and viability. The NaOCl acts as a potent oxidizing agent, generating reactive oxygen species (ROS) that disrupt microbial membranes and damage plant cells when overapplied. Similar oxidative effects have been reported in both plant tissue systems and microbial models[13]. Therefore, careful optimization of exposure time and concentration is essential to ensure efficient disinfection without compromising explant regeneration capacity.

Explants of Roseval pretreated with GA_3+cold displayed the greatest shoot elongation and node number, confirming the synergistic effect of hormonal and thermal cues. GA_3 enhances cell elongation through the activation of expansins and wall-loosening enzymes[23], whereas cold exposure increases cytokinin and auxin levels and reduces abscisic acid accumulation, collectively improving morphogenic competence[18]. The marked differences observed between cultivars emphasize the genotype-specific nature of these responses, consistent with transcriptomic studies revealing distinct hormonal signaling profiles among potato genotypes[21]. Consequently, pretreatment protocols should be adjusted for each cultivar to optimize morphogenesis and regeneration efficiency.

Acclimatization success depended strongly on both substrate composition and genotype. The highest survival rates were recorded on forest soil and sand, likely due to superior root aeration and reduced waterlogging stress. Comparable results were obtained in potato and other vegetatively propagated species, where light-textured media improved root development and water balance[6]. In contrast, mixtures containing large proportions of potting soil resulted in higher mortality, particularly for Charlotte, suggesting limited physiological plasticity during the transition from

in vitro to *ex vitro* conditions. Previous studies attributed such variability to differences in stomatal regulation and antioxidant defense capacity[24]. These findings underscore the need to optimize both substrate properties and humidity management to maximize plantlet survival and promote uniform acclimatization in regenerated potato plants.

CONCLUSION

This study established a reliable and reproducible protocol for *in vitro* regeneration and acclimatization of potato (*Solanum tuberosum* L.) adapted to tropical environments. The combined pretreatment with gibberellic acid (GA_3) and cold (5°C) markedly enhanced bud induction, confirming a synergistic interaction between hormonal and thermal cues in dormancy release and sprout development. Optimal surface disinfection using NaOCl or $Ca(ClO)_2$ at 1% active chlorine for 8 minutes achieved effective sterility while maintaining explant viability. During acclimatization, substrates composed of sand or forest soil supported the highest survival rates, emphasizing the role of substrate texture and aeration in *ex vitro* establishment. Overall, the results highlight the necessity of adjusting pretreatment, disinfection, and acclimatization conditions to the physiological characteristics of each cultivar.

The integrated protocol developed in this work provides a practical framework for local seed potato production under tropical conditions and offers a valuable contribution toward improving yield stability and food security in West Africa.

SIGNIFICANCE STATEMENT

This study provides a reliable and reproducible *in vitro* regeneration protocol for potato cultivars adapted to tropical regions, addressing the critical gap in local seed production systems. By optimizing pretreatment, disinfection, and acclimatization, it enables the mass propagation of healthy, vigorous plantlets, supporting regional food security, enhancing crop productivity, and offering a sustainable strategy for potato cultivation in West Africa.

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