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Research Article

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Posted Date: February 11th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8711263/v1>

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Additional Declarations: No competing interests reported.

Aseptic establishment and *in vitro* germination of *Theobroma cacao* L.: Redox strategies to overcome recalcitrance

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Key Message: This study achieves 100 % asepsis and 86.25 % germination in *T. cacao* by neutralizing the cytotoxic quinone cascade through a predictive redox-stabilized framework, overcoming recalcitrance barriers for elite germplasm mass micropropagation.

Keywords: bioactive modulating agents, genotype-environment interaction, redox microenvironment, recalcitrance, phenolic oxidation

Abstract

In vitro cultivation of *Theobroma cacao* L. poses critical challenges, primarily due to the recalcitrant nature of its seeds and the high susceptibility of its tissues to phenolic oxidation. This research evaluated the effect of bioactive modulating agents (cysteine, ascorbic acid, and activated charcoal) and genotypic factors on *in vitro* germination. By employing an optimized surface disinfection methodology, 100 % asepsis was achieved, significantly surpassing historical records of contamination-related losses (up to 63 %). Germination efficiency reached 86.25 %, being significantly influenced by the Genotype × Environment interaction, where the 'Ocumare 61' cultivar demonstrated superior morphogenic competence. Statistical analysis validated robust mathematical models (R^2_{adj} of 73.77 % for germination and 81.05 % for oxidation), confirming that supplementation with activated charcoal effectively neutralized the cytotoxic quinone cascade through physical adsorption—a primary limiting factor in the 'Ocumare 60' cultivar. Response surface analysis identified the optimal performance in the M3 treatment with 'Ocumare 61'. We conclude that asepsis control and the stability of the redox microenvironment are decisive conditions for unlocking the germinative potential of cacao seeds. These findings establish a predictive framework that strengthens protocols for mass micropropagation and the conservation of elite germplasm.

Abbreviations

AA: Ascorbic acid

32 **AC:** Activated charcoal
33 **GA₃:** Gibberellic acid
34 **G × E:** Genotype × Environment
35 **MS:** Murashige and Skoog (1962) basal medium
36 **PPO:** Polyphenol oxidase
37 **SDW:** Sterile distilled water

38 **Introduction**

39 Cocoa (*Theobroma cacao* L.) is a perennial crop and a commodity of transcendental socioeconomic importance
40 worldwide, serving as the economic pillar for numerous nations across West Africa, South America, and
41 Southeast Asia (Otekunrin 2025). However, the escalating global demand stands in stark contrast to critical
42 production challenges, such as phytosanitary vulnerability and the low productivity of high-value genotypes
43 (Kongor et al. 2024). To mitigate these hurdles, efficient propagation systems are required to facilitate rapid
44 clonal multiplication and the management of elite germplasm. Since conventional seed propagation
45 compromises the exploitation of genetic gains due to gametic segregation, the adoption of advanced
46 biotechnological methods has become an unavoidable necessity (Henao et al. 2024).

47 Within this context, plant biotechnology based on *in vitro* culture emerges as a pivotal technological platform
48 for cocoa sustainability. This methodological framework allows for the overcoming of the species' biological
49 limitations, ensuring mass micropropagation and the strategic conservation of elite germplasm (Potter et al.
50 2024). By guaranteeing the genotypic stability required for plant breeding programs, *in vitro* culture succeeds
51 in surpassing the physiological and genetic constraints inherent to conventional sexual propagation (Henao et
52 al. 2024). Under this premise, the success of *in vitro* cocoa propagation programs depends on the establishment
53 of primary cultures, with seminal germination being the initial critical point (Potter et al. 2024). This phase
54 faces a formidable biological barrier: the recalcitrant nature of the seed. Its extreme sensitivity to desiccation
55 and the accelerated loss of viability impose physiological challenges that severely limit access to elite
56 germplasm (de Oliveira et al. 2025; Nieves-Orduña et al. 2023). Consequently, optimizing seed germination
57 under controlled conditions represents a fundamental strategy for preserving the embryo's ontogenetic integrity
58 and ensuring the efficiency of micropropagation protocols.

59 Despite the use of fresh seeds, the *in vitro* establishment of cocoa is hindered by persistent microbial
60 contamination. Its control through conventional sterilizing agents is not only often ineffective but also causes
61 phytotoxicity that degrades the cellular architecture, nullifying the explant's responsiveness (de Oliveira et al.
62 2025). Nevertheless, the most significant challenge is the exudation and oxidation of phenolic compounds,
63 processes that severely compromise explant viability. The tissues exhibit a release of secondary metabolites

which, upon being oxidized by polyphenol oxidases (PPO), generate cytotoxic quinones (Liu et al. 2024). Far from being a superficial phenomenon, the browning of the culture medium represents a physiological problem that triggers a cascade of programmed cellular necrosis. This phenomenon constitutes the primary limiting factor restricting cellular totipotency in woody species, as it directly interferes with the activation of developmental regulatory genes for cellular pattern reprogramming (de Oliveira et al. 2025; Wang et al. 2025).

To mitigate this phytotoxicity, the integration of bioactive modulating agents into the culture microenvironment has been investigated. Activated charcoal stands out as a high-porosity, non-specific adsorbent that sequesters and compartmentalizes toxic metabolites (Liu et al. 2024). Complementarily, ascorbic acid acts as a direct-action antioxidant that neutralizes free radicals and reduces quinones to their precursor forms (Amente and Chimdessa 2021). Despite these properties, a knowledge gap exists regarding the synergistic interaction and the optimal concentrations required to counteract phenolic oxidation in cocoa.

In light of the above, the objective of this research was to determine the effect of exogenous supplementation with cysteine, ascorbic acid, and activated charcoal on the kinetics and efficiency of *in vitro* seed germination in *T. cacao*. It was hypothesized that the individual use and synergistic integration of these agents modulate the redox microenvironment, allowing for a significant reduction in browning and a substantial increase in successful establishment. Ultimately, this study seeks to establish a reproducible methodological framework to strengthen propagation, conservation, and plant breeding programs on a global scale.

Materials and methods

Plant material

Mature, indehiscent fruits of *Theobroma cacao* L., belonging to cultivars Ocumare 60 (C1) and Ocumare 61 (C2), were selected from the germplasm bank of the Institute of Genetics, Faculty of Agronomy, Central University of Venezuela (UCV-FAGRO). The selection was based on strict phytosanitary criteria and adherence to the standardized morphological descriptors established for each clone.

Fruit surface disinfection methodology

Under laboratory conditions, the pods underwent a surface disinfection process starting with mechanical washing using running water and a commercial detergent solution under constant agitation for 30 min. Subsequently, the material was transferred to a Class II horizontal laminar flow hood to initiate the chemical disinfection protocol.

Initially, three washes with sterile distilled water (SDW) were performed to remove detergent traces. Surface disinfection was carried out by immersing the fruits in 70 % (v/v) ethanol for 10 min, followed by triple clarification with SDW. Next, chemical sterilization was performed using a 6 % sodium hypochlorite (NaClO)

solution for 10 min. The protocol concluded with three successive rinses with SDW to ensure the total removal of residual oxidizing agents prior to fruit opening and seed extraction.

Excision and conditioning of explants

Fruit opening was performed by means of a transverse incision using sterile surgical instruments. Subsequently, the seeds were extracted, followed by the mechanical removal of the mucilage and the manual detachment of the testa using dissection forceps and precision scalpels.

Culture media and conditions

Seeds from cultivars C1 and C2, with the testa removed, were inoculated into Murashige and Skoog basal medium (MS, 1962), supplemented with 30 g·L⁻¹ of sucrose. For each cultivar, four treatments were evaluated (Table 1) to determine the effect of additives on germination kinetics and the incidence of browning.

The pH of the media was adjusted to 5.7 ± 0.1 using NaOH or HCl (1.0 N) prior to the addition of 2.65 g·L⁻¹ of Phytigel®. The media were sterilized by autoclaving at 121 °C and 103.4 kPa (15 psi) for 20 min. Sowing was performed individually in 120 mL glass jars containing 25 mL of culture medium.

Incubation conditions

Following inoculation, cultures were incubated in a growth chamber (Infitek, Model ICB-L450B) at 25 °C and 70 % relative humidity. During the germination phase, explants were maintained in total darkness; this scotoperiod was essential to prevent the light-induced oxidation of phenolic compounds and to promote the elongation of the embryonic axis under controlled conditions.

Germination percentage

Germination was monitored daily over a period of 21 days, establishing the emergence of the radicle with a length exceeding 2 mm as the criterion for success (Potter et al. 2024). The final germination percentage was determined following the methodology of Vera et al. (2025), applying the following equation:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of inoculated seeds}} \times 100$$

Apparent oxidation percentage

To determine the incidence of browning, the degree of darkening of the samples and the culture medium was evaluated following the treatment. The analysis was based on a three-level visual scale, adapted from the four grades established by Holst et al. (2025).

This characterization was based on a visual ordinal scale that identifies chromatic changes derived from the release of exudates from the explant into the culture medium (Fig. 1). Under this criterion, the following descriptors were established:

1. **Low level (no apparent oxidation):** The tissue maintains its turgidity and the natural coloration of the seed. The culture medium remains translucent or retains the original coloration of the basal supplement.
2. **Medium level (moderate oxidation):** The tissue shows localized browning. In the culture medium, the formation of an amber-colored halo is observed at the contact point, with partial diffusion around the explant.
3. **High level (severe oxidation):** The tissue acquires an intense, opaque, and dark brown or black coloration, with an evident loss of turgidity. The culture medium appears darkened, with extensive radial diffusion of exudates that compromises the transparency of the medium.

The quantification of oxidation was performed by classifying the experimental units according to the defined visual scale. Subsequently, the percentage incidence was calculated following the methodology of Xu et al. (2023), using the following equation:

$$\% \text{ Oxidation} = \frac{\text{Number Jars showing oxidation}}{\text{Total number of jars in treatment}} \times 100$$

Experimental Design

The research was conducted using a 2×4 multilevel factorial experimental design with nine (9) replicates per treatment. The study comprised a total of 80 *in vitro* culture experimental runs. Two primary input factors were evaluated:

1. **Factor A (medium):** The MS basal medium served as the control (*M1*), alongside three additional variants (*M2*, *M3*, and *M4*), as detailed in Table 1.
2. **Factor B (cultivar):** Two cacao genotypes were assessed: *C1* and *C2*.

The germination percentage and apparent oxidation percentage were defined as the response variables. Table 2 summarizes the input factors, their respective levels, and the codification used within the experimental design. Data analysis was performed using Statgraphic Centurion XVI (free version). All statistical calculations were conducted at a 95 % confidence level.

Results

Aseptic establishment

During the *in vitro* establishment phase, no incidence of microbial contamination was detected (0 %). This total absence of contaminating agents facilitated the development of an axenic culture from the onset of the process. Consequently, a 100 % explant viability rate was achieved under the specific conditions of the applied protocol. The sterility of the cultures was maintained throughout the evaluation period, allowing for an objective observation of the morphogenic response without interference from surface or endogenous pathogens.

Germination efficiency

After 21 days of *in vitro* culture, the average germination rate was 86.25 %. A significant Genotype $\times$ Environment ($G \times E$) interaction was observed across the different treatments. Specifically, cultivar *C1* achieved its maximum germination efficiency (100 %) in the M1 medium (control). In contrast, cultivar *C2* expressed its highest morphogenic potential when cultured in the M3 medium. The detailed germination data, categorized by treatment and cultivar, are summarized in Table 3.

Statistical analysis and predictive modeling

The Pareto chart (Fig. 2) identifies Factor B and the two-way interaction between Factor A and Factor B as statistically significant variables ($p < 0.05$) for the *in vitro* germination of cacao seeds. The $A \times B$ interaction exerts the most substantial influence on the response variable, greatly exceeding the significance threshold. Factor A, when evaluated independently, lacks a significant main effect, as its impact is intrinsically contingent upon the specific cultivar utilized.

To further explore this interaction, the estimated response surface for germination percentage (Fig. 3) reveals a pronounced slope and curvature. The region of maximum yield is localized toward the coded value of -1 for Factor B, while the highest germination percentage shifts toward positive coded values (+1) for the environmental factor, identifying the M3 medium as the optimal chemical environment. The peaks of maximum physiological response converge at these optimal factor levels.

The behavior of the system is described by the following fitted mathematical model:

$$\text{Germination (\%)} = 83.5007 - 0.7235A + 4.7211B + 1.8548A^2 + 12.6688AB$$

(Where A = Culture Medium and B = Cultivar)

Dynamics of apparent phenolic oxidation

The control of phenolic oxidation was a determining factor during the *in vitro* establishment phase. Overall analysis revealed that 68.75 % of the samples exhibited low oxidation levels (Table 4). However, this outcome was highly dependent on the culture medium formulation.

Media M3 and M4, which included a combination of cysteine, activated charcoal, and ascorbic acid, achieved absolute control (100 %) of browning in both cultivars (C1 and C2), maintaining oxidation at the "low" level (no apparent browning). In contrast, the basal media (M1 and M2) revealed differential susceptibility between genotypes. Cultivar C1 exhibited the highest propensity for oxidation in these media, with 70 % to 80 % of explants showing medium to high browning levels. Conversely, C2 demonstrated an intermediate tolerance, with 50 % of the explants maintaining low oxidation levels even in the absence of antioxidants.

Statistical analysis and predictive modeling of phenolic oxidation

The Analysis of Variance (ANOVA) for the percentage of apparent oxidation, represented in the Pareto chart (Fig. 4), confirms that both Factor A and Factor B are statistically significant. Furthermore, the $A \times B$ interaction was significant ($p < 0.05$), indicating that the oxidative process is jointly determined by the genotype and the chemical environment.

The estimated response surface (Fig. 5) exhibits a planar trend. The region of maximum oxidation is localized at the coded value -1 for Factor B (C1) and -1 for Factor A (M1). The convergence of these factors at their minimum levels results in the highest physiological stress response, as visualized in the upper-left vertex of the surface.

The behavior is described by the following adjusted mathematical model:

$$\text{Oxidation (\%)} = 27.8628 - 33.9622A + 3.5548B + 4.0205A^2 + 5.5114AB$$

(Where A = Culture Medium and B = Cultivar)

The model achieved an adjusted coefficient of determination (R_{adj}^2) of 81.05 %, indicating a high explanatory power for the observed variability. Furthermore, all ANOVA assumptions were strictly validated: normality of residuals (Shapiro-Wilk test, $p > 0.05$), independence (Durbin-Watson test, no autocorrelation), and homoscedasticity (graphical analysis of predicted vs. residuals).

Discussion

Success of the disinfection protocol and explant selection

The 0 % contamination rate observed in this study represents a significant improvement over the typical challenges encountered in the micropropagation of this species. As noted by Okoroafor (2022), *in vitro* viability is frequently compromised by microbial contamination, particularly when explants are sourced from field-grown plants, where microorganisms reside latently on surfaces and within natural openings.

The success of this stage can be attributed to the selection of seeds as the primary source of explants. By extracting tissues from structures protected by the fruit, direct exposure to environmental pathogens—described by Okoroafor (2022) as 'highly detrimental'—was effectively bypassed. This advantage is further evidenced when comparing our results with the findings of Echenique and Calle (2020). In their study using floral organs (staminodes and petals), contamination levels reached up to 98.5%, with even their most effective treatments yielding a 36 % incidence rate.

The marked discrepancy between our results and those of Echenique and Calle (2020) validates the premise that tissue exposure level is a determining factor in establishment success. While floral organs harbor a microbial load that is often difficult to eradicate, the disinfection protocol applied to the fruit in this research effectively neutralized both surface and endogenous threats. This ensured that the subsequent germinative potential and morphogenic response were a direct result of the genotype $\times$ medium composition interaction, free from the metabolic competition or phytotoxicity typically associated with microbial interference.

Morphogenic competence and G $\times$ E interaction

The germination efficiency recorded in this study (86.25 %) aligns with the findings of Potter et al. (2024) regarding the high morphogenic competence of *T. cacao* explants, even under minimal culture conditions and in the absence of growth regulators. However, our results significantly exceed the 69.2 % germination rate reported by those authors. This divergence suggests that the specific disinfection protocol and technical management implemented in this trial optimized the biological transition toward the germinative phase, surpassing existing benchmarks for the species.

The differential behavior between cultivars supports the premise of Sivachandran et al. (2017) and Vera et al. (2025), who argue that *in vitro* formulations must be genotype-specific. For C1, the maximum response in the control medium suggests that its morphogenic success depends primarily on the effectiveness of asepsis rather than nutritional supplementation. Conversely, the growth promotion observed in C2 upon the addition of GA₃ in the M3 medium coincides with the report by Marca et al. (2024), who observed that this phytohormone optimizes tissue response during the *in vitro* establishment of Bolivian cacao. In this context, GA₃ acts as a biochemical trigger that facilitates both the formation of undifferentiated tissue and the stimulation of root emergence in specific genotypes.

The reduced response of C1 under the same nutritional conditions (M3) further supports the contention that the genetic profile is the primary modulator of endogenous hormonal metabolism. This intrinsic variability is consistent with Sivachandran et al. (2017), who established that in *T. cacao*, the genotype is the determining factor governing morphogenic competence, as it conditions tissue sensitivity to both internal and external hormonal stimuli.

Genotype-dependent sensitivity and hormonal homeostasis

The statistical model and the response surface analysis establish that *in vitro* germination is governed by the genetic background of the plant material and its specific interaction with the chemical composition of the medium. This $G \times E$ interdependence aligns with recent studies on *T. cacao* regeneration, which highlight morphogenesis as a genotype-linked phenomenon. In this regard, de Oliveira et al. (2025) argue that the intrinsic morpho-anatomical characteristics of the plant material dictate its regenerative potential, which is subsequently modulated by the physicochemical environment. This is further supported by Marca et al. (2024) and Potter et al. (2024), who demonstrate that germination efficacy results from the biological complementarity between genetic potential and specific nutritional protocols rather than the use of enriched media alone (Henao et al. 2023; Osorio et al. 2022).

A notable finding in this study is the response pattern of cultivar C2, which exhibited superior intrinsic germination capacity and vigor even under limiting conditions. Of particular interest, although GA₃ is a potent inducer of germination widely used in *in vitro* protocols, its exogenous supplementation did not exert a significant effect among the evaluated treatments. This suggests that endogenous gibberellin levels in C2 are sufficient to trigger germination, rendering additional supplementation redundant.

According to Castro-Camba et al. (2022), plant growth regulation depends on precise hormonal homeostasis; thus, the saturation of basal levels restricts the response to external regulators. This observation is consistent with Zhumanova et al. (2024), who emphasize that sensitivity to growth regulators depends on genetic potential. Specifically, the inherent hormonal robustness of C2 obviates the need for chemical supplementation to achieve high germination rates. Consequently, these findings suggest that the universal standardization of culture media is insufficient. Instead, the optimization of propagation protocols must be tailored to the metabolic capacity and genetic profile of each cultivar to effectively process and respond to nutritional and hormonal stimuli (de Oliveira et al. 2025).

Synergistic effect of antioxidants and adsorbents on oxidative stress

The total inhibition of browning in media M3 and M4 underscores the efficacy of a combined antioxidant strategy in mitigating phytotoxicity. The inclusion of ascorbic acid and cysteine was fundamental, as these compounds act as potent reducing agents that convert quinones back to phenols before they polymerize into melanins. As reported by Liu et al. (2024), ascorbic acid neutralizes reactive oxygen species (ROS) generated during explant excision, while cysteine inhibits PPO activity through the formation of stable thiolated conjugates.

Complementing this biochemical action, activated charcoal serves as a porous adsorbent matrix that sequesters toxic metabolites and phenolic exudates released into the medium. This prevents the feedback inhibition of explant development caused by phytotoxicity (Amente and Chimdessa 2021; Liu et al. 2024). While the use of activated charcoal is a recognized strategy for woody plants to create a microenvironment conducive to

morphogenesis, its implementation requires a balanced approach to avoid the non-selective adsorption of essential nutrients and growth regulators (Devi et al. 2024).

The synergy observed between these agents is consistent with the findings of Liu et al. (2024) and Amente and Chimdessa (2021), who argue that an antioxidant complex enhanced by an adsorbent is vital for ensuring embryo viability in recalcitrant species. This dual-action approach—preventing quinone formation while eliminating residual phenols—effectively neutralizes the differential release of phenols observed in C1.

The high sensitivity of C1 in basal media (M1 and M2) suggests higher PPO enzymatic activity or a greater density of phenolic compounds compared to C2. This biochemical divergence validates the necessity of modified media; the combined action of ascorbic acid, cysteine, and activated charcoal ensures culture viability regardless of genotypic specificity, promoting morphogenesis by reducing cellular toxicity (Devi et al. 2024; Permadi et al. 2023).

Model validation and factor influence on oxidative stability

The high R_{adj}^2 value (81.05 %) and the validation of ANOVA assumptions confirm that the proposed mathematical model is a robust tool for predicting oxidative behavior in *T. cacao* establishment. The significance of the $A \times B$ interaction demonstrates that phenolic oxidation is not a random occurrence but a predictable response conditioned by the nature of the exogenous bioactive agents and the intrinsic susceptibility of the cultivar.

The concentration of maximum oxidation at the -1/-1 coded levels (M1 and C1) highlights the critical vulnerability of certain genotypes when cultured in media lacking antioxidant supplementation. In this context, the model effectively quantifies the "protective" effect of the additives in M3 and M4, where the negative coefficient for Factor A in the equation mathematically represents the reduction in oxidation levels. This statistical evidence reinforces the necessity of moving away from standardized basal media toward specialized formulations that consider the metabolic specificities of the plant material.

Conclusions

This study establishes a highly efficient and reproducible protocol for the *in vitro* establishment of *Theobroma cacao* L., achieving 100 % explant viability and an average germination rate of 86.25 %. Our results demonstrate that total control of microbial contamination and phenolic oxidation is contingent upon the strategic selection of protected explants (seeds) and the synergistic application of an antioxidant complex (ascorbic acid, cysteine, and activated charcoal).

Statistical modeling through response surface methodology confirmed that *in vitro* morphogenesis in cacao is governed by a significant $G \times E$ interaction. While cultivar Ocumare 60 showed high germinative potential in

basal media, Ocumare 61 required specific nutritional and hormonal supplementation (GA₃) to reach maximum efficiency. Furthermore, the lack of significant response to exogenous GA₃ in Ocumare 61 suggests an endogenous hormonal homeostasis that obviates the need for traditional chemical triggers, highlighting the necessity of genotype-specific formulations.

Ultimately, this research provides a predictive mathematical framework that reduces empirical "trial and error" in cacao micropropagation. These findings reinforce the shift toward customized biotechnological protocols that account for the metabolic and genetic individuality of the plant material, ensuring the successful scaling of elite cacao cultivars for sustainable agricultural development.

Author Contributions conceptualization DM-V; methodology DM-V, MA, MC-C, GC-B, EP, and DS; validation & formal analysis: DM-V, MA, MC-C, DS, EP and GC-B; investigation (experimental work): MA, EP, DS; resources: DM-V, EP, AQ and DC; writing – original draft preparation: DM-V, MA and GC-B; writing – review & editing: DM-V, GC-B, EP, DS, AQ, and DC. All authors have read and agreed to the published version of the manuscript.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement: not applicable.

Ethical statement: not applicable.

Funding This research was funded by the National Fund for Science, Technology, and Innovation (FONACIT), through the "Cacao Genetic Improvement" project, grant no. 2023PGP238.

Acknowledgements The authors extend their gratitude to the Institute of Genetics of the Faculty of Agronomy at the Central University of Venezuela (UCV) for providing the cacao plant material.

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386

387 **Table 1** Composition of culture media (treatments)

Treatment	Composition
M1	MS medium without supplementation
M2	MS + 3 mg·L ⁻¹ GA ₃ + 100 mg·L ⁻¹ Cysteine + 0.5 g·L ⁻¹ AA
M3	MS + 3 mg·L ⁻¹ GA ₃ + 100 mg·L ⁻¹ Cysteine + 1 g·L ⁻¹ AC
M4	MS + 3 mg·L ⁻¹ GA ₃ + 100 mg·L ⁻¹ Cysteine + 0.5 g·L ⁻¹ AA + 1 g·L ⁻¹ AC

MS: Murashige and Skoog basal medium; **GA₃:** Gibberellic acid; **AA:** Ascorbic acid;
AC: Activated charcoal.

Table 2. Factors, levels, and codification of the 2×4 multilevel factorial experimental design with 9 replicates.

Input factors	Codes and levels	Response Variables
A	-1 (M1);	Germination (%) Apparent oxidation (%)
(culture medium)	-0.33 (M2);	
	1 (M3);	
	0.33 (M4);	
B	-1 (C1);	
(cultivar)	1 (C2)	

M1: Medium 1; **M2:** Medium 2; **M3:** Medium 3; **M4:** Medium 4; **C1:** Cultivar 1 (Ocumare 60); **C2:** Cultivar 2 (Ocumare 61).

Table 3. *In vitro* germination percentage (%) of *T. cacao* seeds from cultivars Ocumare 60 (C1) and Ocumare 61 (C2) after 21 days of culture.

Cultivar	Treatment	Rep.	N° GS	% G
C1	M1	10	10	100
	M2	10	8	80
	M3	10	6	70
	M4	10	7	70
C2	M1	10	9	70
	M2	10	9	90
	M3	10	10	100
	M3	10	10	90
TOTAL	8	80	69	86,25

Rep.: Replications; **N° GS:** Number of germinated seeds; **%G:** germination percentage.

Table 4. Percentage (%) of *in vitro* apparent phenolic oxidation in *T. cacao* seeds from cultivars Ocumare 60 (C1) and Ocumare 61 (C2) after 21 days of culture under different treatments.

Cultivar	Treatment	Rep.	Percentage (%) apparent phenolic oxidation		
			Low Level	Medium Level	High Level
C1	M1	10	30	40	30
	M2	10	20	50	30
	M3	10	100	0	0
	M4	10	100	0	0
C2	M1	10	50	50	0
	M2	10	50	30	20
	M3	10	100	0	0
	M3	10	100	0	0
TOTAL	8	80	68,75	21,25	10
Rep.: Replications					

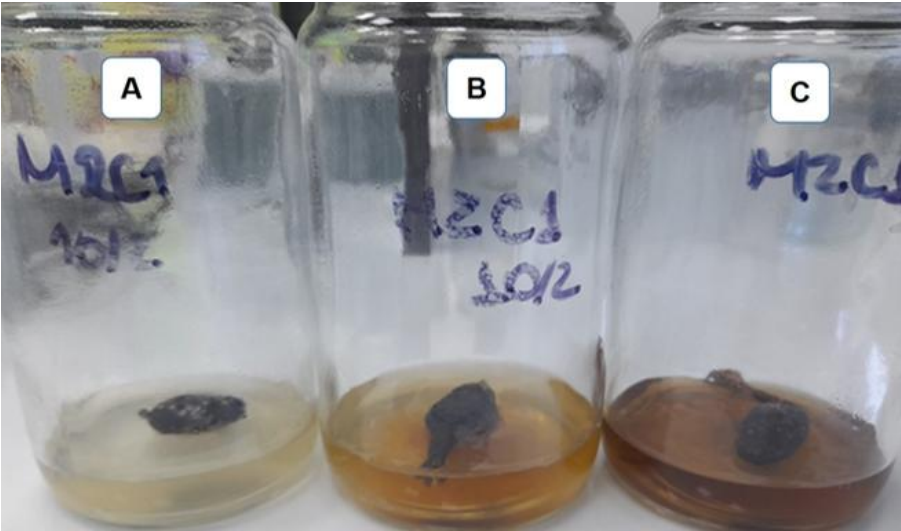


Figure 1. Visual scale of apparent oxidation in *in vitro* cultured cocoa seeds. Levels were categorized according to the degree of tissue browning and exudate diffusion in the medium: A) Low level (control: no oxidation), B) Medium level (moderate oxidation), and C) High level (severe oxidation).

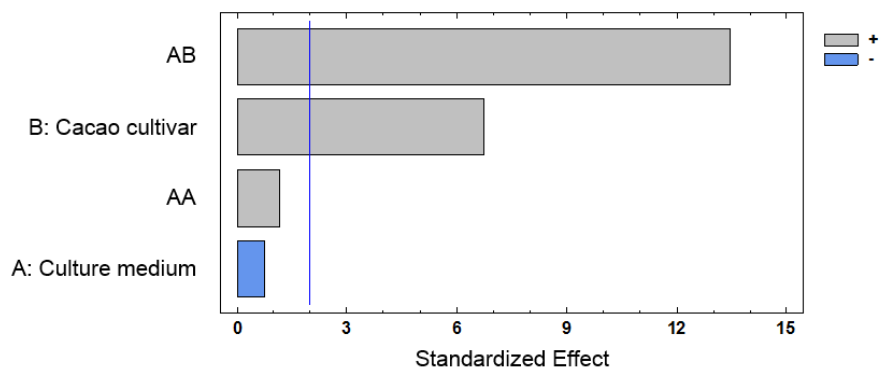


Figure. 2 Standardized Pareto chart for germination percentage (%).

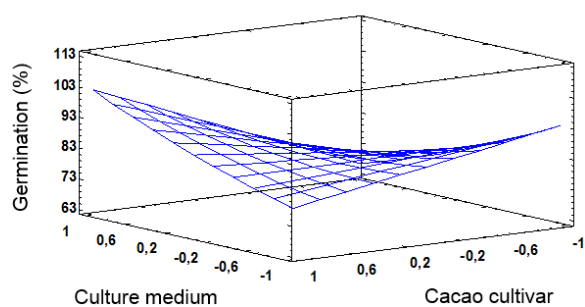


Figure 3. Estimated response surface for germination percentage.

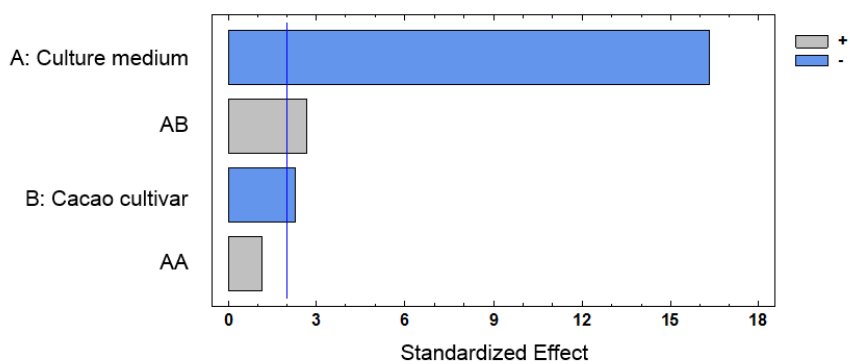


Figure 4. Standardized Pareto chart for the percentage (%) of apparent oxidation.

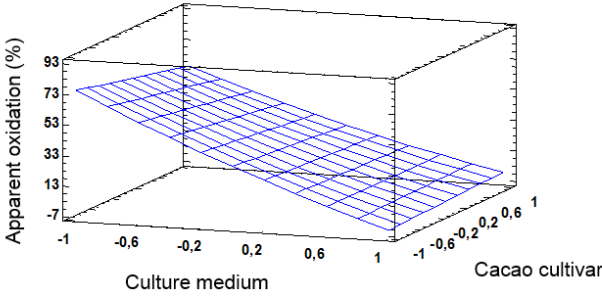


Figure 5. Estimated response surface for the percentage (%) of apparent oxidation.