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Article

Seed Nanopriming with ZnO and SiO₂ Enhances Germination, Seedling Vigor, and Antioxidant Defense under Drought Stress: Implications for Water Use Efficiency in Jalapeño Pepper

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Abstract: Drought stress is a critical constraint limiting seed germination and seedling establishment in field crops such as jalapeño pepper (*Capsicum annuum* L.). Nanopriming, a seed enhancement technique using nanoparticle suspensions, has emerged as a sustainable approach to improve water-use efficiency during early development. This study evaluated the effects of zinc oxide (ZnO, 100 mg·L⁻¹), silicon dioxide (SiO₂, 10 mg·L⁻¹), and their combination (ZnO + SiO₂), stabilized with chitosan, on the germination performance and stress tolerance of four commercial jalapeño hybrids under mannitol-induced drought conditions (0%, 15%, and 30%). Seeds were primed for 12 hours, and physiological, morphological, and biochemical responses were measured. Nanopriming significantly improved germination percentage, speed, and seedling vigor under both moderate (15%) and severe (30%) osmotic stress. SiO₂ enhanced chlorophyll content and germination rate under 15% stress, while ZnO promoted proline and flavonoid accumulation under 30%. The combined ZnO + SiO₂ treatment delivered the most consistent improvements across all traits and stress levels. Multivariate analysis revealed clear treatment-specific and drought-dependent response patterns, emphasizing the role of osmolytes and antioxidant activity. These results highlight the potential of nanopriming as a low-cost, scalable strategy to enhance early-stage drought resilience and promote efficient water use in jalapeño cultivation under semi-arid conditions.

Keywords: Drought-stress; germination; jalapeño pepper; nanopriming; seedling; SiO₂; ZnO

1. Introduction

Drought is one of the most limiting abiotic factors affecting seed germination and early seedling establishment, significantly reducing agricultural productivity in arid and semi-arid regions [1-4]. Under water-deficit conditions, plants experience several physiological disruptions, including decreased water potential [5], excessive accumulation of reactive oxygen species (ROS) [6], and reduced germination rate, seedling vigor, and growth [7-8]. Water is essential for plant metabolism and structure, comprising approximately 85% of the fresh biomass of plants [9].

In experimental settings, mannitol is frequently used to simulate drought by lowering the osmotic potential of the germination medium, allowing the assessment of plant responses to water stress under controlled conditions [10]. In crops such as jalapeño pepper (*Capsicum annuum* L.), water

stress at early stages reduces emergence rate, vigor, and photosynthetic capacity [11]. Jalapeño belongs to the Solanaceae family and is a nutritionally rich crop with significant antioxidant compounds including capsaicinoids, flavonoids, and vitamins A, C, and E [12-13]. Mexico is the second-largest producer worldwide, concentrating over 50% of its national production in the northern states—semi-arid zones where water scarcity is a critical constraint [14-15]).

Improving seedling establishment under drought conditions is crucial to increasing water productivity. Among emerging seed enhancement technologies, seed nanopriming has shown promise. This technique involves soaking seeds in aqueous suspensions of mineral nanoparticles (NPs) at specific concentrations and durations, improving hydration kinetics, antioxidant activity, and seedling vigor under stress conditions [16-17]). Studies with ZnO and SiO₂ nanopriming have reported enhanced germination and early growth in cereals such as wheat and maize under water stress [2, 18-19].

Physiological and biochemical indicators such as proline, phenolic compounds, flavonoids, and soluble sugars are commonly used to evaluate stress responses. These metabolites are involved in osmotic adjustment, membrane stabilization, and ROS scavenging, playing central roles in plant adaptation to drought [20-22].

However, there is limited research on the efficacy of ZnO and SiO₂ nanopriming in commercial jalapeño pepper varieties, especially under mannitol-induced water stress. Therefore, this study aimed to evaluate the effects of seed nanopriming with ZnO and SiO₂ on germination, early growth traits, and oxidative metabolism in *Capsicum annuum* L. under both optimal and drought-simulated conditions. The outcomes of this study may pave the way for the implementation of nanopriming as a viable pre-sowing strategy to improve seed performance under water-deficit conditions, contributing to more sustainable and climate-resilient jalapeño pepper cultivation in semi-arid agroecosystem.

2. Results

2.1. Germination Parameters

Seed germination was significantly influenced by nanopriming treatments, drought stress levels, and jalapeño pepper varieties ($p \leq 0.05$), as shown in Table 1. Treatments were coded using a combination of the variety abbreviation, treatment number, and stress level (see Table 2). For example, MT215 corresponds to the Mixteco variety treated with ZnO (Treatment 2) under 15% drought stress.

Under optimal conditions (0%), treatments such as IMT10, FT10, IMT20, FT20, IMT30, and IMT40 achieved 100% germination, while IT10 (Ideal + hydropriming) showed the lowest germination (75%). Nanopriming with SiO₂ (IT30) resulted in the shortest average germination time (2.33 ± 0.08 days) and the highest germination rate ($0.429 \pm 0.015 \text{ day}^{-1}$), outperforming FT30 (4.13 ± 0.14 days and $0.242 \pm 0.009 \text{ day}^{-1}$, respectively).

Table 1. Germination parameters of four commercial varieties of jalapeño pepper under different priming treatments.

Under	Treatme	Vari	Cod	Germi	Germin	Mean	Mean	Germin	Uncert
0%	T1	MT1	7.00	87.50 ±	3.334 ±	0.300 ±	30.0 ±	1.43 ±	MT10
	Hydropr iming	IT10	6.00	75.00 ±	3.444 ±	0.291 ±	29.0 ±	0.97 ±	IT10
		IMT	8.00	100.00	3.458 ±	0.289 ±	28.9 ±	0.98 ±	IMT10
		FT1	8.00	100.00	3.458 ±	0.289 ±	28.9 ±	0.98 ±	FT10
		MT2	7.00	87.50 ±	3.191 ±	0.313 ±	31.4 ±	1.40 ±	MT20
	T2	IT20	7.00	87.50 ±	3.334 ±	0.300 ±	30.0 ±	0.90 ±	IT20
		IMT	8.00	100.00	3.583 ±	0.280 ±	28.0 ±	1.12 ±	IMT20
		FT2	8.00	100.00	3.458 ±	0.290 ±	29.0 ±	0.86 ±	FT20
	T3	MT3	7.00	87.50 ±	2.905 ±	0.344 ±	34.4 ±	0.78 ±	MT30
		IT30	7.00	87.50 ±	2.334 ±	0.429 ±	42.9 ±	0.90 ±	IT30
		IMT	8.00	100.00	3.292 ±	0.304 ±	30.4 ±	0.86 ±	IMT30
	SiO2 + Q	FT3	7.66	95.83 ±	4.137 ±	0.242 ±	24.2 ±	1.42 ±	FT30
		MT4	7.66	95.83 ±	3.000 ±	0.334 ±	33.4 ±	1.00 ±	MT40
	T4	IT40	7.00	87.50 ±	3.334 ±	0.300 ±	30.0 ±	0.90 ±	IT40

15%	ZnO + SiO ₂ + Q	IMT	8.00	100.00	3.708	± 0.271	± 27.1	± 1.27	± IMT40
		FT4	6.33	83.33	± 4.095	± 0.244	± 24.4	± 1.42	± FT40
	T1 Hydropriming	MT1	8.00	100.00	2.917	± 0.343	± 34.3	± 0.72	± MT115
		IT11	7.33	91.66	± 3.696	± 0.271	± 27.2	± 1.20	± IT115
		IMT	7.00	87.50	± 3.952	± 0.255	± 25.5	± 1.25	± IMT11
		FT1	8.00	100.00	3.375	± 0.297	± 29.7	± 1.02	± FT115
	T2	MT2	7.00	87.50	± 2.905	± 0.344	± 34.4	± 0.78	± MT215
		IT21	7.66	95.83	± 3.476	± 0.288	± 28.8	± 1.09	± IT215
	ZnO + Q	IMT	7.00	87.50	± 3.334	± 0.300	± 30.0	± 0.90	± IMT21
		FT2	8.00	100.00	3.375	± 0.297	± 29.7	± 0.92	± FT215
	T3	MT3	8.00	100.00	3.042	± 0.329	± 32.9	± 0.54	± MT315
		IT31	8.00	100.00	3.208	± 0.312	± 31.2	± 0.72	± IT315
	SiO ₂ + Q	IMT	6.66	83.33	± 3.595	± 0.278	± 27.8	± 1.36	± IMT31
		FT3	7.00	87.50	± 3.429	± 0.292	± 29.2	± 0.94	± FT315
	T4	MT4	6.00	75.00	± 3.167	± 0.316	± 31.6	± 0.52	± MT415
		IT41	8.00	100.00	3.417	± 0.293	± 29.3	± 0.97	± IT415
	ZnO + SiO ₂ + Q	IMT	8.00	100.00	3.792	± 0.264	± 26.4	± 1.46	± IMT41
		FT4	8.00	100.00	3.458	± 0.289	± 28.9	± 1.14	± FT415
30%	T1 Hydropriming	MT1	7.66	95.83	± 3.268	± 0.307	± 30.6	± 1.03	± MT130
		IT13	8.00	100.00	3.375	± 0.297	± 29.7	± 0.92	± IT130
		IMT	8.00	100.00	4.292	± 0.233	± 23.3	± 1.82	± IMT13
		FT1	7.00	87.50	± 4.095	± 0.244	± 24.4	± 1.36	± FT130
	T2	MT2	8.00	100.00	3.708	± 0.270	± 27.0	± 1.44	± MT230
		IT23	6.33	79.16	± 3.992	± 0.253	± 25.3	± 1.17	± IT230
	ZnO + Q	IMT	8.00	100.00	4.000	± 0.251	± 25.1	± 1.45	± IMT23
		FT2	8.00	100.00	3.292	± 0.304	± 30.4	± 0.86	± FT230
	T3	MT3	8.00	100.00	3.625	± 0.276	± 27.6	± 1.12	± MT330
		IT33	7.33	91.66	± 3.690	± 0.272	± 27.2	± 1.22	± IT330
	SiO ₂ + Q	IMT	7.00	87.50	± 3.857	± 0.261	± 26.1	± 1.56	± IMT33
		FT3	7.33	91.66	± 3.958	± 0.253	± 25.4	± 1.62	± FT330
	T4	MT4	8.00	100.00	3.417	± 0.293	± 29.3	± 0.97	± MT430
		IT43	8.00	100.00	3.500	± 0.287	± 28.7	± 1.27	± IT430
	ZnO + SiO ₂ + Q	IMT	6.33	79.16	± 4.436	± 0.226	± 22.6	± 1.18	± IMT43
		FT4	6.33	79.16	± 3.690	± 0.271	± 27.1	± 1.43	± FT430

T1: (Hydropriming); T2: (ZnO + Q); T3: (SiO₂ + Q); T4: (ZnO + SiO₂ + Q), and water stress levels (0%, 15%, and 30%). Statistical analysis was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparisons (p ≤ 0.05). Superscript letters indicate significant differences.

A

B

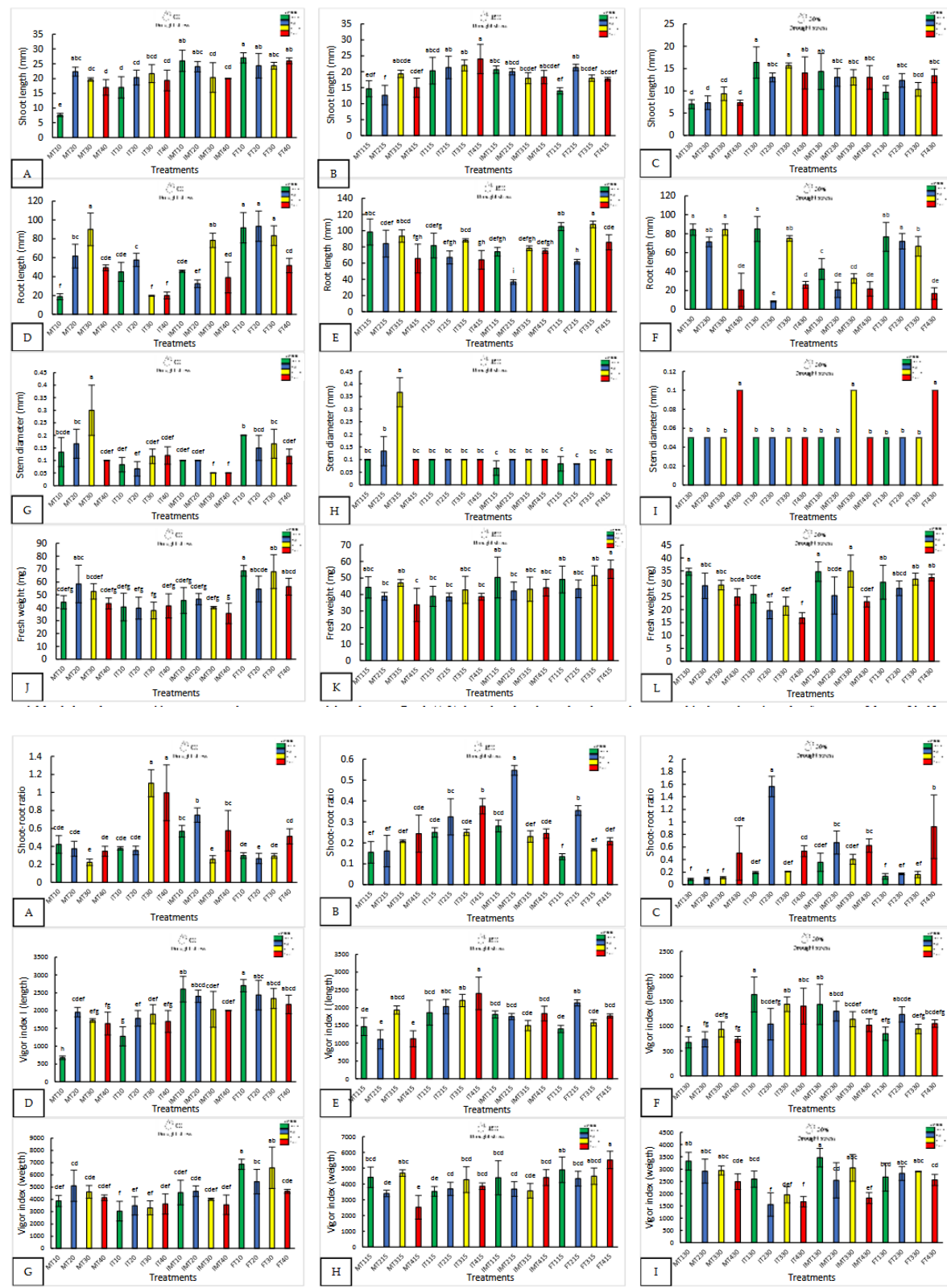
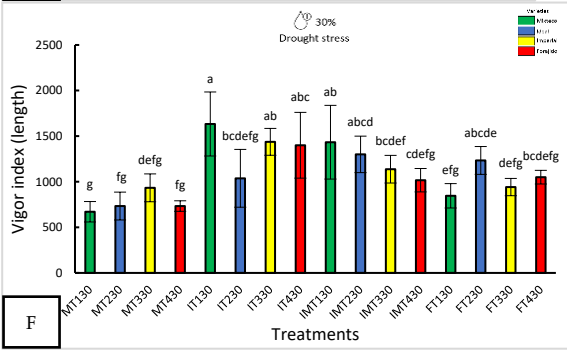
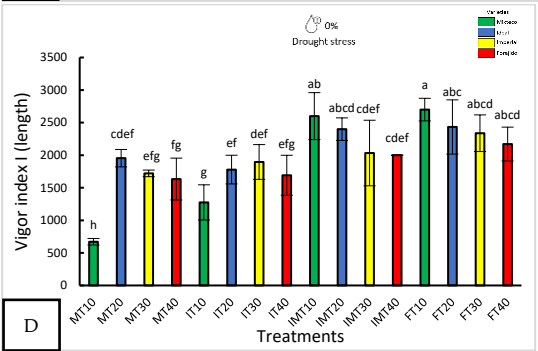
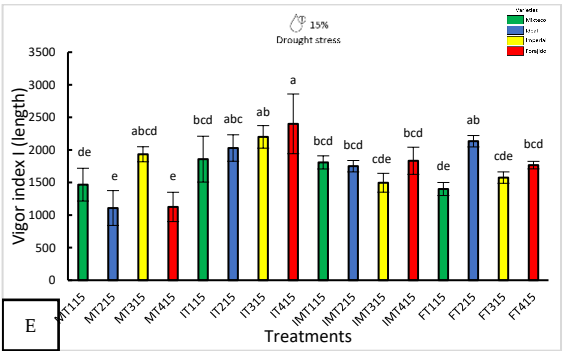
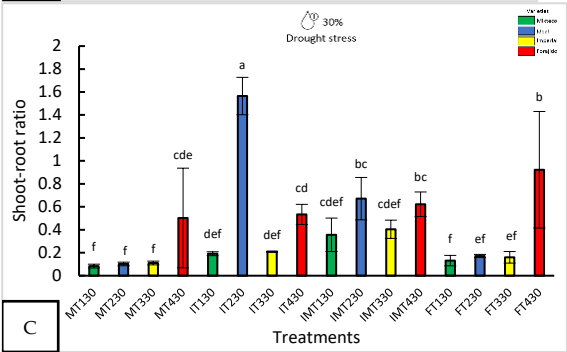
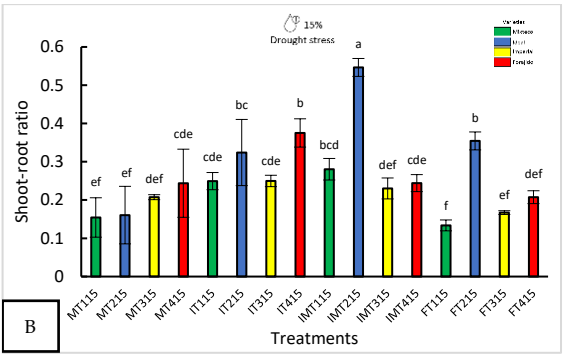
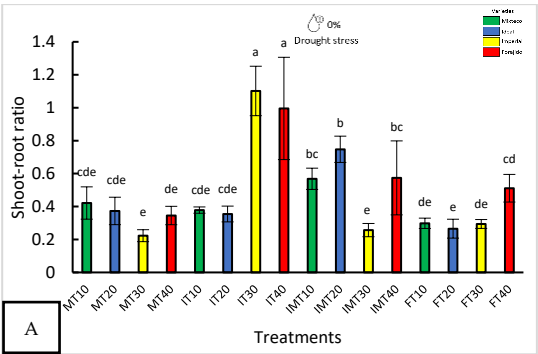


Figure 1. Morphological response of four varieties under nanoprimering and drought stress. Panels (A-L) show shoot length, root length, stem diameter, and fresh weight in four jalapeño varieties (Mixteco, Ideal Imperial and Forajido) under three drought levels (0%, 15% and 30%) and four treatments : T1 (Hydropriming), T2 (ZnO +Q), T3 (SiO₂ + Q), and T4 (ZnO + SiO₂ + Q). Bars represent mean ± SD. Different letters indicate significant differences (Tukey HSD, $p \leq 0.05$).



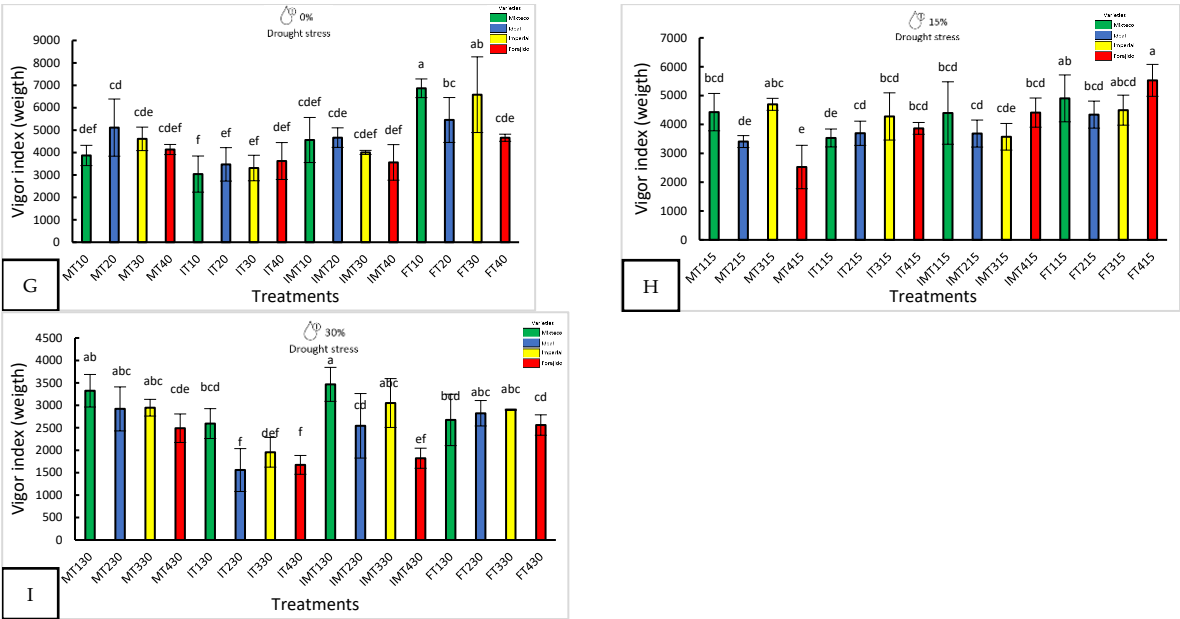


Figure 2. Morphological response of four varieties under nanopriming and drought stress. Panels (A-I) show shoot-root ratio, Vigor Index I and Vigor Index II in four jalapeño varieties (Mixteco, Ideal Imperial and Forajido) under three drought levels (0%, 15% and 30%) and four treatments: T1 (Hydropriming), T2 (ZnO + Q), T3 (SiO₂ + Q), and T4 (ZnO + SiO₂ + Q). Bars represent mean $\pm$ SD. Different letters indicate significant differences (Tukey HSD, $p \leq 0.05$).

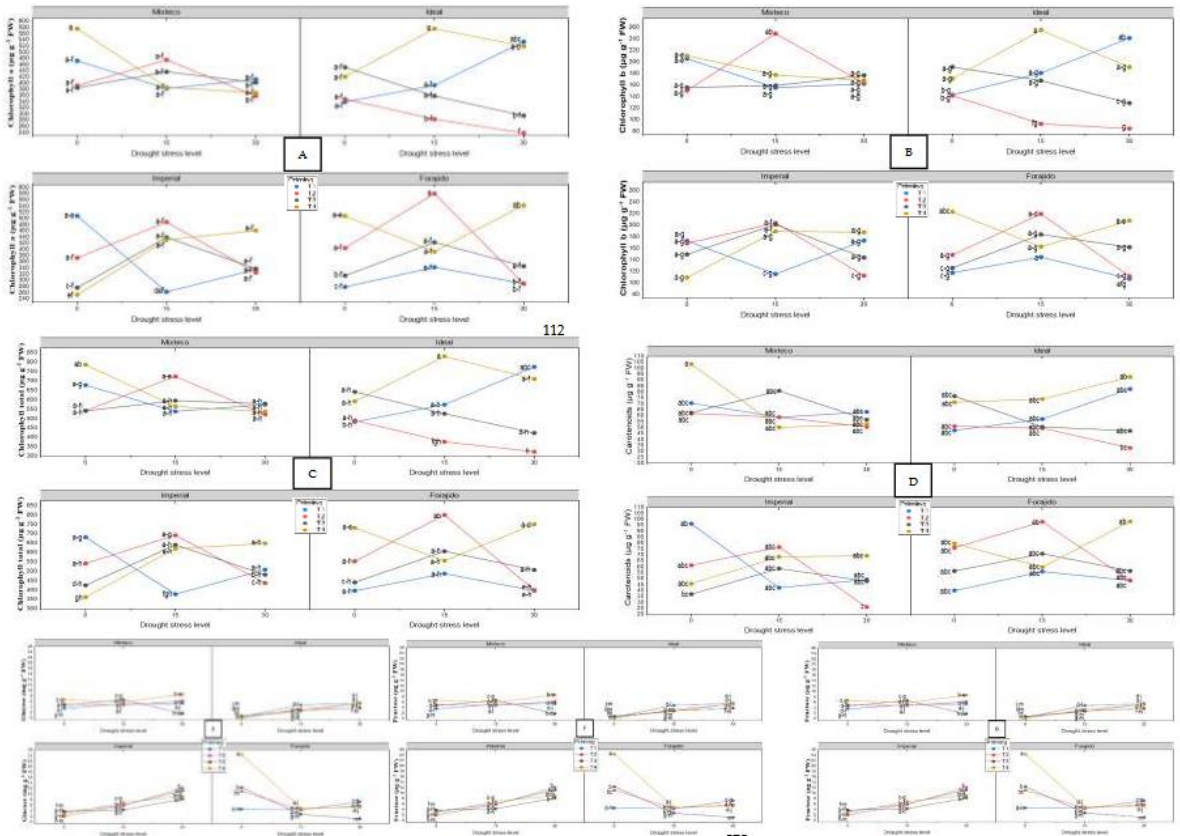


Figure 3. Effect of nanopriming treatments and drought stress levels on the photosynthetic pigments and soluble sugar composition of four jalapeños pepper varieties. Panels (A-G) display the response in the varieties Mixteco, Ideal, Imperial and Forajido under three drought stress levels (0%, 15%, and 30%) and four nanopriming treatments: T1 (Hydropriming), T2 (ZnO + Q), T3 (SiO₂ + Q), and T4 (ZnO + SiO₂ + Q). The variables assessed were: (A) Chlorophyll (a); (B) Chlorophyll (b); (C) Chlorophyll total; (D) Glucose; (E) Fructose; (F) Sucrose. Each data point represents the mean $\pm$ standard deviation. Different letters indicate statistically significant differences.

significant differences (Tukey HSD, $p \leq 0.05$). Under severe drought conditions (30%), FT230 and MT330 achieved complete germination, while IMT430 and FT430 showed reduced performance (79.16%). Notably, FT230 also had the lowest uncertainty index (0.86 ± 0.08), indicating greater synchronization in germination events.

2.2. Morphological Parameters

Nanopriming significantly improved seedling morphology, especially under water stress (Figure 1 and Figure 2). With 0% drought, FT10 recorded the longest shoot length (27 ± 0.91 mm) and the highest fresh weight (68.67 ± 4.16 mg), while FT20 and MT30 showed the longest roots. MT30 also had the largest stem diameter (0.3 ± 0.1 mm), three times larger than IMT30.

With 15% stress, FT415 (Forajido + ZnO + SiO₂) produced the highest fresh weight of the seedlings, while IT415 had the longest shoots. FT315 showed the most extensive root growth (87.5 ± 2.88 mm). The highest shoot-to-root ratio was observed in IMT215 (0.54), while FT115 had the lowest (0.13), indicating a variation in resource allocation among the treatments.

Under 30% water stress, IT130 had the longest shoots (16.3 ± 3.1 mm), and MT330 and MT130 had the longest roots. IMT330 and MT130 also had the highest fresh weights (>34 mg). IT230 showed the highest shoot-to-root ratio (1.56), suggesting preferential shoot development under extreme drought conditions.

2.3. Biochemical Parameters

2.3.1. Photosynthetic Pigments

Pigment levels (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were analyzed using a two-way ANOVA (water stress level x priming treatment) performed separately for each jalapeño variety, followed by Tukey's HSD test ($p \leq 0.05$).

Chlorophyll and carotenoid content varied significantly between treatments and stress levels (Figure 3A–D). In the absence of water stress (0%), the Mixteco variety with the ZnO + SiO₂ treatment (MT40) had the highest values of total chlorophyll (787.26 ± 31.79 $\mu\text{g}\cdot\text{g}^{-1}$ PF) and carotenoids (103.49 ± 8.25 $\mu\text{g}\cdot\text{g}^{-1}$ PF). Under moderate stress (15%), IT415 (Ideal + ZnO + SiO₂) achieved the highest total chlorophyll content (831.64 ± 173.63 $\mu\text{g}\cdot\text{g}^{-1}$ PF). Under severe stress conditions (30%), IT130 (Ideal + hydropriming) showed the highest concentrations in all pigment variables, indicating remarkable stress tolerance in that specific treatment.

2.3.2. Soluble Sugars

Glucose, fructose, and sucrose levels were analyzed using two-way ANOVA (drought stress level x priming treatment) performed separately for each jalapeño variety, followed by Tukey's HSD test ($p \leq 0.05$). Sugar content significantly increased in response to both drought stress and nanopriming treatments (Figure 3E–G).

Under non-stress conditions (0% drought), the Forajido variety treated with ZnO + SiO₂ (FT40) accumulated the highest total sugar content (24.39 $\text{mg}\cdot\text{g}^{-1}$ FW). At 15% drought stress, IMT415 (Imperial + ZnO + SiO₂) exhibited significantly elevated levels of glucose, fructose, and sucrose. Under 30% drought stress, IMT230 (Imperial + ZnO + Q) recorded the highest sugar accumulation.

2.3.3. Free Proline

Proline content showed significant variation between treatments and drought levels ($p \leq 0.05$), as shown in Figure 4A. Nanopriming with ZnO, especially when combined with SiO₂, resulted in substantial proline accumulation.

Under stress-free conditions (0%), FT40 (Forajido + ZnO + SiO₂) recorded the highest proline level (790.29 ± 254.64 $\mu\text{g}\cdot\text{g}^{-1}$ FW), more than 50 times higher than IT10 (Ideal + hydropriming), which had the lowest value. With 15% drought, FT415 showed the highest accumulation (410.36 ± 104.89 $\mu\text{g}\cdot\text{g}^{-1}$ FW), while IT315 had the lowest (22.64 ± 18.88 $\mu\text{g}\cdot\text{g}^{-1}$ FW). Under severe stress (30%), IT430 led all treatments with 448.50 ± 189.00 $\mu\text{g}\cdot\text{g}^{-1}$ FW, compared to only 53.08 ± 8.61 $\mu\text{g}\cdot\text{g}^{-1}$ FW in IT130. These findings confirm the role of proline in osmotic adjustment and indicate that ZnO and ZnO + SiO₂ priming effectively promote stress tolerance through osmoprotective mechanisms.

2.3.4. Total Phenolic Content (TPC)

Significant differences in TPC were detected between treatments and stress levels (Figure 4B). With 0% stress, FT40 (Forajido + ZnO + SiO₂) had the highest TPC (55.28 ± 5.89 mg GAE·g⁻¹ FW), almost 4.8 times higher than MT10 (Mixteco + Hydropriming), which had the lowest content (11.50 ± 0.41 mg GAE·g⁻¹ FW).

With 15% stress, IT115 (Ideal + hydropriming) showed the highest TPC (26.41 ± 3.68 mg GAE·g⁻¹ FW), while FT215 (Forajido + ZnO) had the lowest (10.81 ± 0.89 mg GAE·g⁻¹ FW). With 30% drought, IT430 maintained the highest phenolic content (51.40 ± 6.35 mg GAE·g⁻¹ FW), representing a 3.2-fold increase compared to IT130 (16.08 ± 10.45 mg GAE·g⁻¹ FW). These data suggest that the combination of ZnO and SiO₂ increases phenol accumulation, especially under severe drought conditions.

2.3.5. Total Flavonoid Content (TFC)

The TFC also showed differences depending on treatment and stress (Figure 4C). Under optimal conditions, FT40 recorded the highest TFC (21.97 ± 8.40 mg QE·g⁻¹ FW), almost 100 times higher than IT40 (0.22 ± 0.13 mg QE·g⁻¹ FW). Under 15% stress, FT115 had the highest TFC (9.00 ± 1.90 mg QE·g⁻¹ FW), while IT315 had the lowest (0.14 ± 0.67 mg QE·g⁻¹ FW). With 30% drought, FT430 retained the highest flavonoid levels (16.20 ± 5.03 mg QE·g⁻¹ FW), 70 times higher than those of FT230. These results highlight the improvement in flavonoid biosynthesis caused by ZnO and SiO₂, especially in combined treatments.

2.3.6. DPPH Antioxidant Activity

Antioxidant activity, evaluated by DPPH radical scavenging, varied significantly among treatments and stress levels ($p \leq 0.05$) (Figure 4D). With 0% stress, FT40 showed the highest activity (15.72 ± 1.01 mg TE·g⁻¹ FW), indicating a strong baseline antioxidant potential. Under 15% stress, IT415 showed the highest DPPH activity (5.61 ± 0.01 mg TE·g⁻¹ FW), while under 30% drought, IT430 again led the way (11.53 ± 0.63 mg TE·g⁻¹ FW). These results suggest that nanoprimering with ZnO + SiO₂ effectively stimulates antioxidant defense systems over a wide range of drought intensities.

2.4. PCA and Heat Map Analyses

2.4.1. PCA and Heatmap Under 0% Stress

Under stress-free conditions, principal component analysis (PCA) explained 55.23% of the total variance between treatments. PC1 accounted for 33.56% and was mainly associated with morphological traits such as shoot and root length, fresh weight, and chlorophyll content. PC2 explained 21.67% of the variance and was related to biochemical parameters such as proline, total flavonoid content (TFC), and DPPH activity (Figure 5A). This separation indicates that, even in the absence of drought, nanoprimering influenced growth and antioxidant responses differently.

The heat map (Figure 5B) revealed strong positive correlations between total chlorophyll content and phenolic content (TPC), as well as DPPH activity ($r > 0.87$), indicating a functional relationship between photosynthetic pigments and antioxidant capacity under stress-free conditions.

2.4.2. PCA and Heatmap under 15% Stress

With a water stress of 15%, PCA explained 36.61% of the total variation. PC1 grouped morphological traits such as germination speed, shoot length, fresh weight, and vigor indices. PC2 was mainly driven by variables related to antioxidants, such as proline, TFC, and DPPH (Figure 5C). This suggests that nanoprimering under moderate drought conditions triggers an integrated physiological and biochemical response.

The corresponding heat map (Figure 5D) highlighted a strong clustering of proline, DPPH, and TFC, with correlation coefficients above 0.85. This indicates a coordinated response of osmoprotective and antioxidant pathways under moderate stress.

2.4.3. PCA and Heatmap under 30% Stress

Under severe water stress conditions (30% mannitol), PCA accounted for 40.74% of the total variance. PC1 captured most of the variation and was dominated by osmoprotective and antioxidant variables—proline, soluble sugars, TPC, and DPPH activity—reflecting a metabolic shift toward biochemical protection. PC2 was largely associated with photosynthetic pigments (chlorophyll a, b, and carotenoids) (Figure 5E).

The heat map (Figure 5F) showed very strong correlations between proline, DPPH, and TPC ($r > 0.90$), supporting the hypothesis that responses to oxidative and osmotic stress are closely coordinated under high water stress. In addition, chlorophylls and carotenoids showed a high mutual correlation ($r = 0.89$), indicating synchronized photoprotective mechanisms.

These multivariate patterns demonstrate that the response to drought stress varies depending on the treatment and severity level, and that priming with ZnO and SiO₂, especially in combination, is consistently associated with stress resistance traits.

2.5. Radar Chart: Multivariate Comparison by Drought Level

A radial graph (Figure 6) was used to visualize the comparative performance of nanopriming treatments on 15 key variables and three stress levels (0%, 15%, and 30%). Each axis represents a trait, and each line corresponds to a treatment: ZnO (green), SiO₂ (red), and ZnO + SiO₂ (blue).

Under optimal conditions (0%), ZnO led in carotenoids, shoot length, and total chlorophyll, while SiO₂+ZnO outperformed the others in phenols and DPPH activity. SiO₂ alone improved fresh weight and sugar content, indicating osmotic benefits even in stress-free environments.

With 15% stress, SiO₂ treatment consistently outperformed the others in germination speed, DPPH activity, phenol accumulation, and fresh biomass. ZnO was favorable for vigor and shoot-root balance, while the combined treatment showed a balanced profile in all biochemical and morphological parameters.

With 30% stress, the combination of ZnO + SiO₂ showed superior performance in total chlorophyll, DPPH activity, and TPC, indicating a strong photoprotective and antioxidant response. ZnO improved root development and germination, while SiO₂ stood out in proline accumulation and TPC, reinforcing its role in osmotic adjustment and biochemical defense.

Overall, the radar chart illustrates that nanopriming combined with ZnO and SiO₂ consistently elicited synergistic responses under drought conditions, offering a robust strategy for improving drought resistance in jalapeño pepper seedlings.

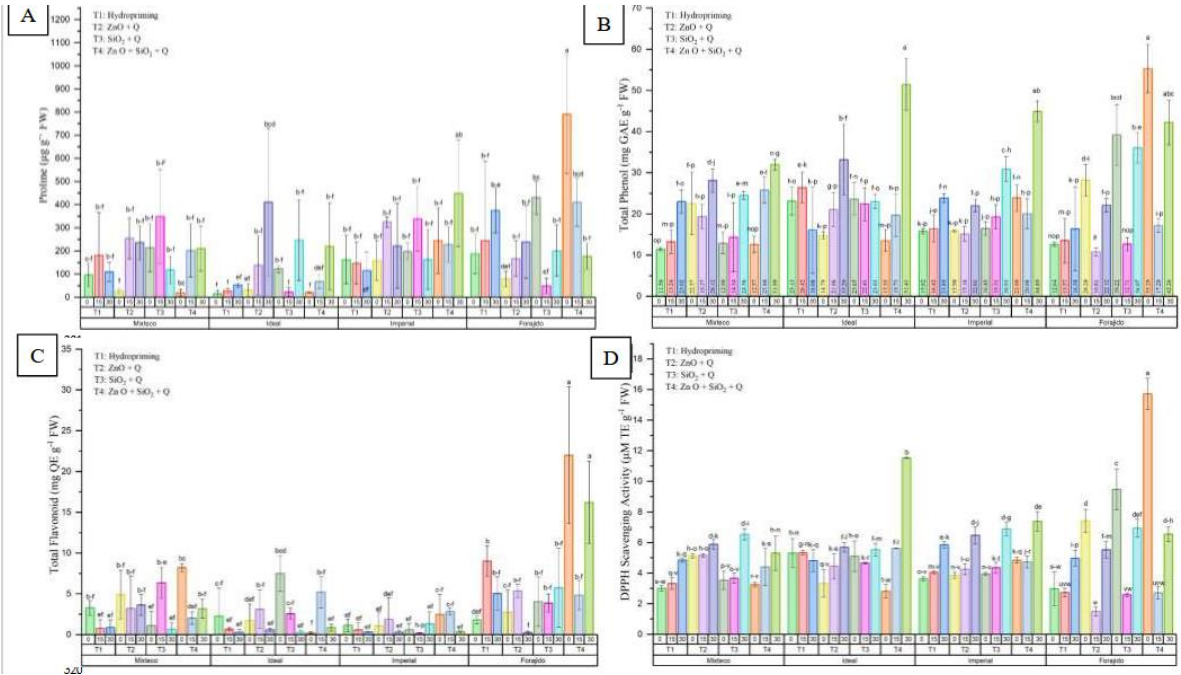


Figure 4. Biochemical responses in four jalapeño pepper varieties under different nanopriming treatments and drought stress levels. The panels show: (A) Free proline content ($\mu\text{g g}^{-1} \text{FW}$), (B) Total phenolic content (mg GAE

g^{-1} FW), (C) Total flavonoid content (mg QE g^{-1} FW), and (D) DPPH radical scavenging activity (mg TE g^{-1} FW). Bars represent the mean $\pm$ standard deviation for each variable. The treatments applied were T1 (Hydropriming), T2 ($\text{ZnO} + \text{Q}$), T3 ($\text{SiO}_2 + \text{Q}$), and T4 ($\text{ZnO} + \text{SiO}_2 + \text{Q}$), across three drought stress levels (0%, 15%, and 30%) in the varieties Mixteco, Ideal, Imperial, and Forajido. Letters above the bars indicate significant differences between treatments according to a Tukey HSD test ($p \leq 0.05$).

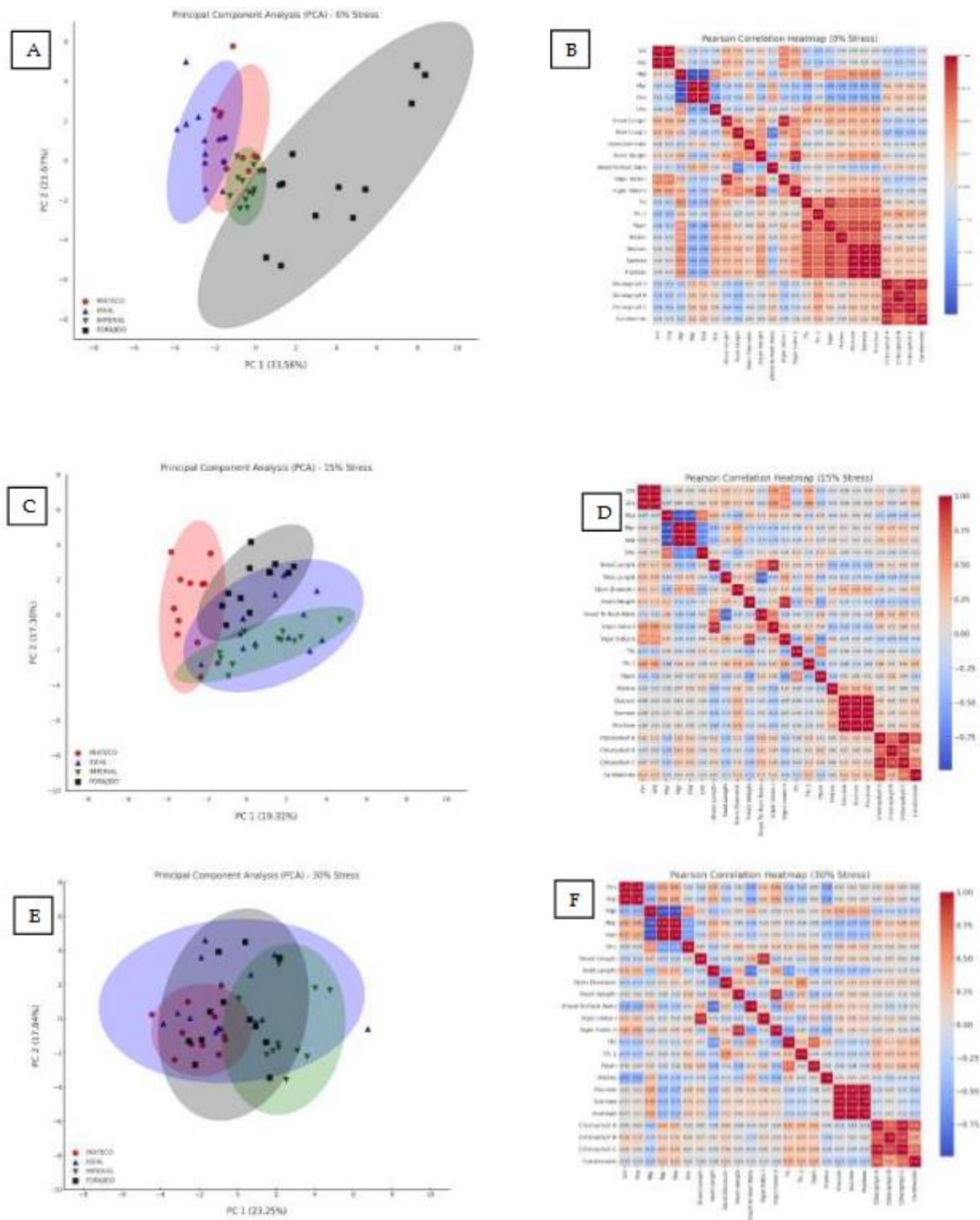


Figure 5. Principal Component Analysis (PCA) and Pearson Correlation Heatmaps under Different Water Stress Levels. Panels (A), (C), and (E) represent the PCA biplots of morphophysiological and biochemical traits evaluated in four jalapeño pepper varieties (MIXTECO, IDEAL, IMPERIAL, and FORAJIDO) under 0%, 15%, and 30% water stress conditions, respectively. The ellipses represent the distribution and clustering of each variety based on the first two principal components. Panels (B), (D), and (F) show the corresponding Pearson correlation heatmaps for the same variables and stress levels. Positive correlations are shown in red and negative

in blue, with the intensity indicating the strength of the correlation. These analyses highlight the multivariate relationships and changes in trait associations as water stress intensifies.

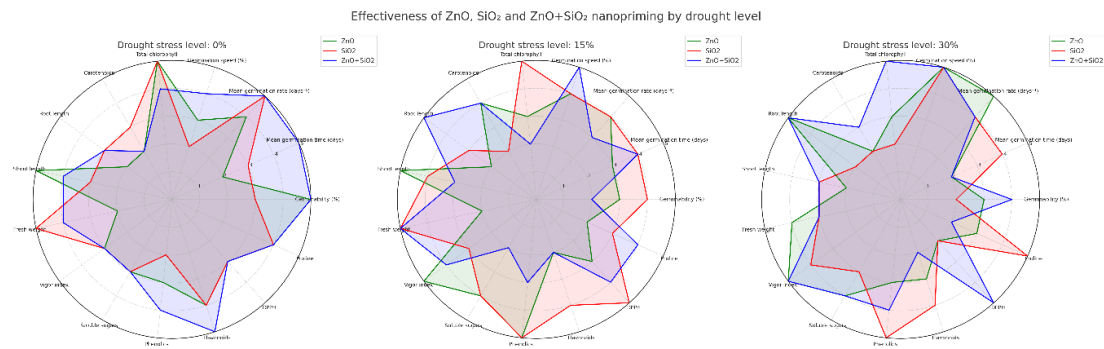


Figure 6. Radar chart comparing ZnO (green), SiO₂ (red), and ZnO+SiO₂ (blue) nanopriming in jalapeño under 0%, 15%, and 30% drought stress. Fifteen variables were evaluated. ZnO+SiO₂ showed the best overall performance at 30% stress, while SiO₂ stood out at 15%. Combined treatments improved both growth and antioxidant responses under drought.

3. Discussion

This study demonstrated that nanopriming with ZnO, SiO₂, and their combination effectively improved germination dynamics, early growth, and biochemical responses in jalapeño peppers (*Capsicum annuum* L.) under different levels of drought stress. The responses observed depended on the type of nanoparticle, the severity of water stress, and the plant variety, highlighting the relevance of interactions between variety, environment, and nanomaterials.

These results support previous findings indicating that nanopriming can improve crop performance under abiotic stress by triggering physiological and biochemical responses that enhance water use efficiency, osmotic regulation, and oxidative stress management [16, 21-22].

Under optimal conditions (0% water stress), priming with ZnO and SiO₂ significantly improved germination parameters. Treatments such as IMT20, FT20, and IMT40 promoted faster and more uniform germination compared to the hydroprimed control. This improved performance can be attributed to increased α -amylase activity and gibberellin signaling, which accelerate starch degradation and reserve mobilization, favoring early seedling development [22]. The significant reduction in average germination time and uncertainty in IT30, a SiO₂ priming treatment, suggests early metabolic activation potentially related to membrane stabilization and increased aquaporin activity [23-24]. Even without water stress, the activation of these physiological mechanisms through nanopriming likely creates a “priming state,” which may confer long-term resilience.

Under moderate water stress (15% mannitol), nanoprimed seeds in treatments such as FT415, IT315, and MT315 maintained high germination rates—up to 100% in FT415 and IT315—and showed vigorous root elongation, shoot development, and biomass accumulation. These responses suggest that nanopriming facilitates the maintenance of cell turgidity, osmotic balance, and early stress signal transduction. This is consistent with previous findings in chickpeas, tomatoes, and okra, where ZnO and SiO₂ NPs improved drought tolerance by maintaining root growth and physiological function [25-28]. Specifically, ZnO-treated seedlings showed improved root architecture, likely due to increased mitotic activity and root system plasticity, which allowed for greater water uptake under conditions of limited availability [29]. In addition, ZnO contributed to the balance between shoots and roots, while SiO₂ improved osmotic regulation and preserved tissue integrity by reinforcing cell wall stability and hydration dynamics [30].

Under severe water stress (30% mannitol), although biomass accumulation decreased, treatments such as FT430, IT430, and MT230 maintained high germination rates and showed strong accumulation of osmoprotectants and antioxidant compounds. This shift from growth promotion to biochemical defense enhancement indicates a physiological balance that prioritizes survival over expansion, a well-established adaptive response under abiotic stress [31]. It is noteworthy that proline

levels increased dramatically, especially in ZnO+SiO₂ treatments, confirming its role as an osmoprotectant and ROS scavenger [20].

Proline also showed strong correlations with phenols and DPPH activity, consistent with stress responses in *Mentha pulegium* and corn [32-33]. Similar synergistic effects of silicon and zinc NPs in improving osmoprotectant accumulation and stress mitigation in wheat under salinity have been reported, where the joint application of proline with Zn and Si NPs significantly improved proline content, antioxidant activity, and plant growth (Abd-Elzaher et al., 2025). These results reinforce the concept that, under conditions of intense drought, nanoprimering modulates seedling metabolism towards greater biochemical resilience rather than growth, particularly through the synergistic effects of ZnO and SiO₂.

Similarly, total phenolic content (TPC) and total flavonoid content (TFC) increased significantly in nanoparticle treatments, particularly in FT40 and IT430. These secondary metabolites play an essential role in non-enzymatic antioxidant defense, redox regulation, and cell protection under stress. Their accumulation under optimal and drought conditions suggests that nanoprimering may induce a mild oxidative signal that preactivates antioxidant pathways, improving the plant's preparedness for stress [21]. This state of preparedness was supported by the high DPPH radical scavenging activity in the same treatments, indicating a robust antioxidant response. These findings are consistent with previous studies showing that ZnO and SiO₂ NPs improve phenolic biosynthesis and antioxidant potential in crops such as potato and wheat under water-limited conditions [34-36]. Furthermore, evidence obtained with NPs based on *Mimusops elengi* extracts also supports their role in stimulating phenol production and ROS detoxification [37]. In line with this, the antioxidant effects observed here are consistent with reports on plant systems treated with pink pepper and citrus extracts under oxidative conditions [38].

Multivariate analyses further confirmed the integrative role of nanoprimering in modulating growth and defense responses under water stress. Principal component analysis (PCA) revealed that, under 0% stress, germination and morphological traits—such as root length, shoot length, fresh weight, and chlorophyll content—had a large weight in PC1, while antioxidant markers such as proline, total flavonoids (TFC), and DPPH activity contributed predominantly to PC2. This clear separation reflects the dual action of nanoprimering in promoting early vigor and activating defense pathways. As drought intensified to 15% and 30% mannitol, the PCA structure changed markedly: osmoprotectants and antioxidants explained most of the variance, indicating a physiological transition toward biochemical prioritization. Similar patterns have been described in chickpea, where PCA-based multivariate selection was driven by biochemical markers under stress conditions [39]. At 30%, variables such as proline and glucose became the main contributors to PC1, highlighting their central role in osmotic adjustment and metabolic reprogramming.

Heat map correlation analysis complemented these findings, revealing strong associations ($r > 0.90$) between proline, DPPH, phenols, and TFC, especially under moderate and severe stress, indicating a closely coordinated antioxidant and osmotic defense network. These trends mirror observations made in *Cicer arietinum* and maize, where ROS scavenging, osmotic balance, and root system architecture (RSA) were crucial for drought adaptation [26, 40]. Under low stress, high correlations between chlorophylls, phenols, and DPPH reflected physiological homeostasis. At 15%, the synergy between TFC and proline with fresh weight suggested metabolic flexibility and maintenance of turgidity. At 30%, increased correlations between sugars and antioxidant compounds revealed a trade-off favoring stress survival over growth, a phenomenon described as an adaptation strategy in plants under drought stress [31].

To better visualize the multidimensional performance of each treatment, a radial graph (Figure 6) was constructed that summarizes 15 variables at all stress levels. This graph provided an intuitive and comprehensive view of the relative strengths of each nanoprimering treatment. With 0% stress, ZnO treatments stood out in photosynthetic pigment content and shoot development, while SiO₂ + ZnO showed superior antioxidant capacity. SiO₂ alone improved fresh weight and sugar content, highlighting its role in osmotic stabilization even in unstressed seedlings. At 15% stress, SiO₂ consistently obtained the best results in germination speed, antioxidant activity, phenol accumulation, and biomass, suggesting strong ROS detoxification and greater water use efficiency.

ZnO was most effective in maintaining the balance between shoots and roots, and the combined ZnO+SiO₂ treatment showed a balanced profile in morphological and biochemical dimensions.

Under severe drought conditions (30%), the superiority of the ZnO+SiO₂ treatment became even more evident. This combined treatment outperformed all others in total chlorophyll, DPPH activity, and phenols, reflecting integrated photoprotective and antioxidant defense. ZnO favored root development and germination under stress, while SiO₂ treatments achieved the highest levels of proline, further reinforcing its osmoprotective role. Thus, the radial graph confirmed the complementarity and potential synergism between these NPs, especially under difficult environmental conditions. It also illustrated the ability of nanopriming to activate different but interconnected response mechanisms depending on the level of drought.

Together, these results underscore the value of nanopriming as a specific, economical, and scalable technology for improving crop performance under drought conditions. In regions such as northern Mexico, where more than 50% of the national jalapeño production occurs and water scarcity is a major constraint [13, 41], the implementation of nanopriming protocols could significantly improve seedling establishment and productivity. Furthermore, the consistency of responses across varieties and stress levels suggests that these technologies could be extended to other crops and production systems.

Together, the evidence indicates that nanopriming with ZnO, SiO₂, and their combination plays a crucial role in improving the physiological and biochemical performance of jalapeño pepper seedlings under drought conditions. These treatments promoted more efficient germination, improved early structural development, and activated antioxidant defense pathways, especially under moderate and severe water deficit conditions. Integrative analysis using a radar chart highlighted the multidimensional advantages of the combined ZnO+SiO₂ treatment, reinforcing its value as a synergistic and promising approach for improving drought resilience during the most vulnerable stages of plant establishment.

4. Materials and Methods

4.1. Experimental Conditions and Plant Material

The experiment was conducted at the Food and Development Research Center (CIAD), Delicias Unit, Chihuahua, Mexico. Four commercial varieties of jalapeño pepper F1 (*Capsicum annuum* L.) were used: Mixteco, Ideal, Imperial, and Forajido. The seeds were visually inspected to ensure uniformity and then disinfected with 4% sodium hypochlorite for 2 minutes, followed by three rinses with sterile distilled water, as described by Pandya et al. [42].

4.2. Nanoparticle Preparation and Priming Treatments

Zinc oxide (ZnO) and silicon dioxide (SiO₂) NPs were synthesized using the coprecipitation method and characterized by transmission electron microscopy (TEM), which confirmed an average size of 50-80 nm (Figure 7). A solution of 100 mg L⁻¹ of each nanoparticle was prepared in triple-distilled water with 0.1% chitosan as a dispersant. The solutions were homogenized by magnetic stirring (60 min) and sonication (15 min), as described by Waqas et al. [22]. The priming treatments consisted of soaking the seeds in 30 ml of each solution for 12 hours at 25 °C in the dark, followed by drying at room temperature for 24 hours. The treatments were coded according to variety (M: Mixteco, I: Ideal, IM: Imperial, F: Forajido), nanoparticle type (T1: hydropriming, T2: ZnO, T3: SiO₂, T4: ZnO + SiO₂) and stress level (10 = 0%, 115 = 15%, 130 = 30%) (see Table 1). These codes were used consistently in data collection, figures, and analysis (see Figures 1–6).

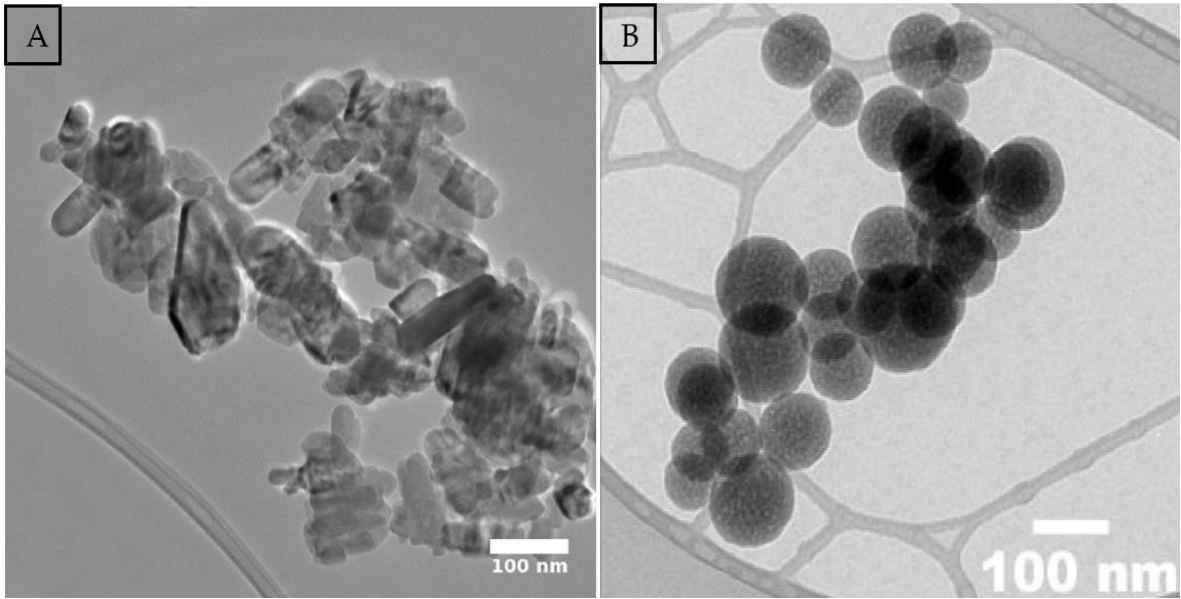


Figure 7. Morphology of the sample by Transmission Electron Microscopy (TEM), (A) ZnO, (B) SiO₂.

4.3. Experimental Design

A completely randomized design (CRD) was used, with a 4 × 4 × 3 factorial arrangement, where: factor A corresponded to four varieties of jalapeño pepper seeds; factor B consisted of four priming treatments; and factor C consisted of three levels of induced water stress. The effects of nanopriming and water stress levels on germination, initial growth, chlorophyll content, total soluble carbohydrates, free proline, total phenols, total flavonoids, and antioxidant capacity were evaluated using the DPPH method (Figure 8). Each analysis was performed in triplicate.

Each experimental unit corresponded to a Petri dish with 8 seeds, considering each seed as a replicate within its respective treatment.

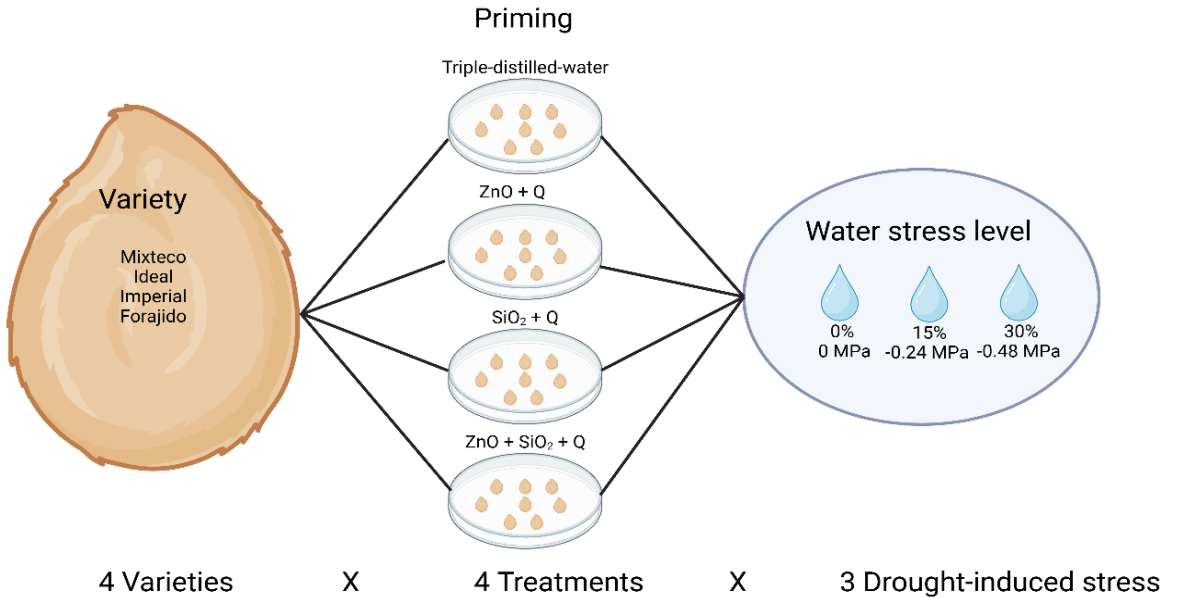


Figure 8. Experimental design with a 4 × 4 × 3 factorial arrangement, evaluating four varieties of jalapeño pepper, four priming treatments and three levels of induced water stress.

4.4. Drought-Induced Stress

Water stress was induced using mannitol at concentrations equivalent to 0% (control), 15% (-0.24 MPa), and 30% (-0.48 MPa), following the method of Ertuğ and Yazıcılar [10]. Germination was carried out in Petri dishes (9 cm in diameter) lined with Whatman No. 4 filter paper moistened with

5 ml of the corresponding mannitol solution. Eight seeds per dish were incubated under controlled conditions ($28 \pm 2\text{ }^{\circ}\text{C}$, 16/8 h light/dark cycle).

4.5. Germination Assay

Germination was monitored daily for 8 days. Seeds were considered germinated when the radicle exceeded 1 mm (Figure 9). Germination indices (percentage, mean germination time, mean germination rate, speed, and uncertainty) were calculated using the GerminaR package [43]. Germination evaluation followed the ISTA [44] guidelines. Results are shown in Table 1.

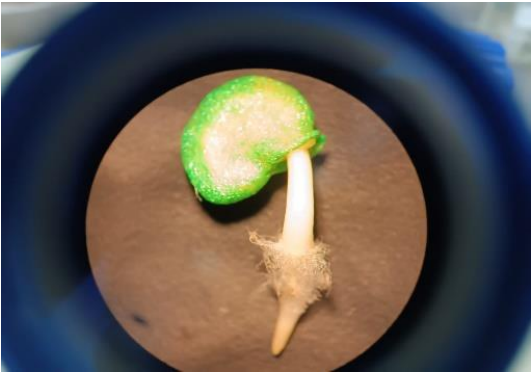


Figure 9. Germination criteria radicle length 1 to 2 mm (Binocular stereo microscope with VE-S4 zoom system (Velab Co., Pharr, Texas, USA).

Table 2. Description of the distribution of the varieties of jalapeño pepper used in the study, the treatments, the level of induced stress, and the code used.

Jalapeño pepper varieties	Treatment NPs + Chitosan	Code		
		Level of osmotic stress		
		0%	15%	30%
Mixteco (M)	(T1) Triple-distilled water Control	MT10	MT115	MT130
	(T2) ZnO 100 mgL ⁻¹ + Q 100 mgL ⁻¹	MT20	MT215	MT230
	(T3) SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	MT30	MT315	MT330
	(T4) ZnO 100 mgL ⁻¹ + SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	MT40	MT415	MT430
Ideal (I)	(T1) Triple-distilled water Control	IT10	IT115	IT130
	(T2) ZnO 100 mgL ⁻¹ + Q 100 mgL ⁻¹	IT20	IT215	IT230
	(T3) SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	IT30	IT315	IT330
	(T4) ZnO 100 mgL ⁻¹ + SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	IT40	IT415	IT430
Imperial (IM)	(T1) Triple-distilled water Control	IMT10	IMT115	IMT130
	(T2) ZnO 100 mgL ⁻¹ + Q 100 mgL ⁻¹	IMT20	IMT215	IMT230
	(T3) SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	IMT30	IMT315	IMT330
	(T4) ZnO 100 mgL ⁻¹ + SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	IMT40	IMT415	IMT430
Forajido (F)	(T1) Triple-distilled water Control	FT10	FT115	FT130
	(T2) ZnO 100 mgL ⁻¹ + Q 100 mgL ⁻¹	FT20	FT215	FT230
	(T3) SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	FT30	FT315	FT330
	(T4) ZnO 100 mgL ⁻¹ + SiO ₂ 10 mgL ⁻¹ + Q 100 mgL ⁻¹	FT40	FT415	FT430

4.6. Morphological Measurements

On day 8, seedlings were evaluated for shoot length, root length, fresh weight, and stem diameter using a digital caliper. Radicle length was measured from the base of the hypocotyl to the apex of the radicle, while plumule (shoot) length was measured from the radicle–hypocotyl junction to the base of the cotyledons [41]. Stem diameter was measured at approximately 2 mm above the root–hypocotyl transition zone. Vigor indices I and II were calculated according to Abdul-Baki and Anderson [45]

Morphological variability between varieties was interpreted using the descriptors proposed by Elizondo-Cabalceta and Monge-Pérez [46]. Data were collected by variety and treatment to evaluate growth performance under different drought stress levels (Figure 2 and Figure 3).

4.7. Biochemical Analyses

Photosynthetic pigments (chlorophyll a, b, total chlorophyll, and carotenoids) were extracted with 99% methanol and quantified spectrophotometrically at 665, 652, and 470 nm, using the equations of Wellburn [47]. The proline content was quantified using the ninhydrin method, using benzene extraction and absorbance at 520 nm. Soluble sugars (glucose, fructose, sucrose) were extracted with 80% ethanol and analyzed using the anthrone method at 620 nm [48].

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method with gallic acid as a standard [49], and the total flavonoid content (TFC) was measured using the AlCl_3 colorimetric method with quercetin as a standard [38]. Antioxidant activity was determined by DPPH radical scavenging [35]. The absorbance for TPC, TFC, and DPPH was measured at 765, 510, and 517 nm, respectively. The data for these biochemical traits are shown in Figures 3 and 4.

4.8. Multivariate and Correlation Analysis

Principal component analysis (PCA) and Pearson correlation matrices were performed using OriginPro 2025 to evaluate relationships between morphological and biochemical traits. PCA biplots were generated separately for each drought level (0%, 15%, 30%) (Figure 5A,C, E), and heatmaps were constructed to visualize correlations between traits and treatments (Figure 5B,D,F) [39]. A radar chart (Figure 6) was created to integrate germination, growth, and biochemical performance of ZnO, SiO_2 , and ZnO+ SiO_2 treatments across the three stress levels, normalizing 15 key variables.

4.10. Statistical Analysis

Each variable was represented by three replicates. Germination parameters were analyzed using the nonparametric Kruskal-Wallis test followed by Dunn's post hoc test ($p \leq 0.05$), due to the non-normal distribution of the data.

For early growth variables and biochemical parameters such as photosynthetic pigments and soluble sugars, the assumptions of normality, homogeneity of variances, and independence were tested. When these assumptions were met, a two-way ANOVA (drought stress level $\times$ priming treatment) was performed separately by variety. Tukey's HSD test ($p \leq 0.05$) was used to determine significant differences between means.

For antioxidant-related variables—total phenols, flavonoids, proline content, and DPPH radical scavenging activity—a three-way ANOVA was conducted to evaluate the effects of priming treatment, drought stress level, and variety, including their interactions. Post hoc comparisons were performed using Tukey's HSD test ($p \leq 0.05$).

All statistical analyses were carried out using SAS® software version 9.0 (SAS Institute Inc., Cary, NC, USA [50]). Figures, bar charts, radar charts, Pearson correlation matrices, and principal component analysis (PCA) plots were generated using OriginPro 2025 (v10.2.0.196).

5. Conclusions

Nanopriming with ZnO, SiO_2 and their combination significantly improved drought tolerance in jalapeño peppers by enhancing germination, seedling vigor, and antioxidant capacity under both moderate and severe water stress conditions. The combined treatment with ZnO + SiO_2 produced the most consistent improvements in physiological and biochemical parameters, indicating a synergistic effect in promoting stress resilience in the early stages. These findings support the integration of nanopriming as a pre-planting strategy to optimize seed performance under water-scarce conditions. By strengthening seedling establishment and improving metabolic responses under drought conditions, this approach contributes to improving water use efficiency and promoting more sustainable water resource management in crop production systems.

Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: E.H.O.-C. and E.S.; data collection: L.U.C.-E., S.P.-Á. and J.J.P.-C.; analysis and interpretation of results: C.C.-M., J.C.A.-P. and E.M.-M.; draft manuscript preparation: E.H.O.-C. and E.S. All authors have read and agreed to the published version of the manuscript.

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