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Calcium phosphate nanoparticles improve growth parameters and mitigate stress associated with climatic variability in avocado fruit

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ABSTRACT

The avocado cv. Hass is one of the most dynamic fruits in the world and is of particular significance in tropical areas, where climate variability phenomena have a high impact on productivity and sustainability. Nanotechnology-based tools could be an alternative to mitigate and/or adapt plants to these phenomena. Our approach was based on identifying changes in temperature and precipitation associated with climate variability in avocado areas in Colombia and proposing mitigation strategies based on the use of nanotechnology. This study had two objectives: (i) to identify variations in temperature and precipitation in avocado-producing areas in Colombia and (ii) to evaluate the effect of calcium phosphate nanoparticles (nano CP) as an alternative to reduce stress in avocados under simulate climatic variability condition. Climatic clusters were determined based on the spatial K-means method and with the climatic temporal series data (1981–2020), a time series analysis we carried out. Later changes in each cluster were simulated in growth chambers, evaluating physiological and developmental responses in avocado seedlings subjected to nanoCaP after adjusting the application form and dose. XRD diffraction shows that the calcium phosphate phases obtained by solution combustion correspond to a mixture of hydroxyapatite and witocklite nanoparticles with irregular morphologies and particle sizes of 100 nm. Three clusters explained ~90% of the climate variation, with increases and decreases in temperature and precipitation in the range of 1-1.4 °C and 4.1–7.3% respectively. The best-fitted time series models were of stationary autoregressive integrated moving averages (SARIMA). The avocado seedlings had differential responses ($P < 0.05$) depending on the clusters, with a decrease in physiological behavior and development between 10 and 35%. Additionally, the nanoCaP reduced the climatic stress ($P < 0.05$) in a range between 10 and 22.5%. This study identified the negative effect of climate variability on avocado seedlings and how nanoCaP can mitigate these phenomena.

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1. Introduction

The avocado (*Persea americana* Mill) cv. Hass is the most cultivated and popular variety worldwide and has notable nutritional benefits and beneficial effects on human health [1,2]. In recent years there has been a sustained increase in planted area and production of Hass avocado [3], especially in tropical areas, where Colombia is one of the most dynamic countries [3,4]. This rapid growth has brought great challenges, such as increasing the productivity, quality, and sustainability of the production system [5–7].

The growing boom in planting this fruit tree has led to new areas being used for cultivation in non-optimal edaphoclimatic conditions, as well as to expansion of these areas to sensitive ecosystems, with implications for systems sustainability and water uses [7–9]. For example, cultivation has been extended to soils that are poor in nutrients, degraded, clayey, have low water supply, and in extreme climates. These conditions have a high impact on the productive and sustainability potential of the species [7,10,11]. In addition, the high climatic variability of tropical conditions is a factor that greatly influences dynamic pest populations, yield, and fruit quality parameters in different planted crops [12–15].

Climate variability refers to variations in the mean or other statistical parameters of the climate variables (i.e., temperature and precipitation) under spatial-temporal dimensions beyond that of individual internal or external weather events [16]. In tropical areas, climate variability is associated with seasonal and non-seasonal phenomena. The Intertropical Convergence Zone (ITCZ) [17,18] is one such seasonal phenomenon. The ITCZ has a high effect on the precipitation regime and its distribution throughout the year [19]. Meanwhile, non-seasonal or interannual variation is mainly associated with ENSO phenomena (El Niño Southern Oscillation) [20,21]. The ENSO- El Niño is generally associated with warm periods and droughts, while the ENSO- La Niña is characterized by cold periods, accompanied by an increase in precipitation [21]. The ENSO (El Niño-La Niña) has high regional variation with regard to duration and intensity [21,22].

The identification of phenomena associated with climate variability in agricultural production areas is crucial to understanding the stress processes generated in plants as a result of changes in climatic variables [14,23]. The rise of the climate change and climatic variability has resulted in a concerning escalation in the occurrence and severity of diverse abiotic stresses, including droughts, heatwaves, cold spells, and floods [24]. These environmental challenges have had detrimental effects on crop productivity and quality, leading to food scarcity and jeopardizing global food security [24–26]. Under the current dynamics of climatic variability and the future implications associated with climate change, the avocado production system is highly vulnerable to phenomena such as droughts, increases in temperature, higher flood frequencies and reduction in water supply [8,14,27]. Likewise, understanding these dynamics will aid the design of adaptation and mitigation strategies for these phenomena [14,28,29].

Temperature increases and precipitation variations linked to the ENSO phenomenon in tropical regions can have a substantial impact on avocado production systems. These changes can result in significant yield reductions, elevated pest populations, and adverse effects on quality parameters [11,14]. In addition, currently the issue of water consumption by this fruit tree is a sensitive topic with potentially serious consequences [10], given that a decrease in the natural water supply through precipitation and increase of transpiration of the plants associated with higher temperatures in some producing regions would mean that the suitability of these zones for avocado production would need to be reconsidered [30]. These antecedents indicate the need to seek alternatives that can mitigate the adverse effects of climatic variability in highly sensitive production systems such as avocado. Among the mitigation strategies for phenomena associated with climate variability such as droughts, the application of calcium (Ca^{2+}) has been reported as an excellent alternative [31,32]. Additionally, the application of phosphorus (P) has been reported as an alternative to mitigate the adverse effect of water deficits and improve physiological and biochemical response parameters [33,34].

Nanotechnology is an emerging smart technology in agriculture and may provide multiple comparative advantages in the search for adaptation and mitigation measures for climate variability in agricultural production systems. In recent years, the potential application of nanotechnology in agriculture has been explored, including the controlled release of fertilizers, antimicrobial activity, fungal activities, disease control, reduction of abiotic stress conditions, and many other functions [35–37]. Recent advances in nanotechnology and its applications in plants may improve crop growth and productivity [37]. The potential effects of nanoparticles (NPs) related with plant growth and reduction of abiotic stress can be associated with an increase in seed germination, root growth, chlorophyll content, photosynthesis rate, and biomass [38]. However, the biological implications depend on their physicochemical properties (as surface tension), concentration, the method of application and events that occur at the molecular level [39]. Normally the application methods are at the level of the roots and foliage. In the case of foliage, the interaction mechanisms of NPs can occur through stomatal or cuticular interactions and the successful entry into the plant will depend on the relationship between their respective size [40,41].

Nanotechnology can improve multiple aspects related to the adverse effects of climate variability and climate change in plants [38]. The most notable applications of NPs related to adaptation and mitigation to these phenomena include the efficient and sustainable use of fertilizers [42–44]; better management of water and biotic and abiotic stress sources [35,36]; and promotion of plant growth and physiological behavior [36,45]. Calcium and phosphorus-based nanoparticles (HAPNps or calcium phosphates) can be used as sources to release ions, and such uses may be a novel strategy. Calcium phosphates are inorganic structures identified as ceramic materials, which are normally used as biomaterials in various engineering fields [46,47]. For example, HAPNps have been used as sources of phosphorus or in the design of controlled release systems for active compounds such as urea, emerging as the next generation of high-performance nitrogen fertilizers [48,49].

Additionally, the HAPNps has been used as a novel calcium phosphates nano-fertilizer with high impart on the plant growth compared to the traditional NPK fertilizer on commercial crops of rosemary (*Rosmarinus officinalis* L.) [50], radish (*Raphanus sativus* L.) [51], tomato (*Lycopersicum esculentum* Mili.) [52], lettuce (*Lactuca sativa* L.) [53], corn (*Zea mays* L.) [54], among others. Overall, the effects of calcium phosphates on plants can be influenced by the concentration of nanoparticles, and plants demonstrate varying responses to calcium phosphates. Therefore, additional research is necessary to comprehend the mechanisms involved in the absorption of calcium phosphates and the potential positive and negative impacts they may have.

No studies have been made of climate variations under tropical conditions in avocado crops or how they affect physiological, growth and development variables of the cv. Hass. Likewise, the behavior of cv. Hass avocado seedlings subjected to stress from increased temperatures and associated reductions in precipitation under the influence of calcium phosphate nanoparticles is not known. Therefore, the objectives of this work were: (i) to forecast variations in precipitation and temperature in avocado-producing areas in Colombia; and (ii) to evaluate the effect of calcium phosphate nanoparticles (nano CP) as an alternative to reduce stress in avocados under simulate climatic variability condition.

2. Materials and methods

2.1. Methodological approach

Our work proposes a methodological framework based on multiple stages in which we aim to fulfill the hypothesis that avocado-producing regions in Colombia exhibit a differential impact on precipitation and temperature variables associated with climate variability. Furthermore, based on the utilization of innovative tools such as nanotechnology, we explore potential mitigation alternatives for these phenomena.

As the first component of analysis and in the pursuit of generating homogeneous climatic zones as a basis for studying climate and the impact of climate variability at regional scales, we conducted a climate characterization based on clustering analysis [55]. Subsequently, for each cluster and based on *in-situ* weather station data, we proceeded to adjust the historical data series (1981–2020) using stochastic time series models. Once the model with the best fit was obtained, we performed a decomposition of the series into its seasonal components, stochastic and deterministic variation (increase and/or decrease) for precipitation and temperature variables. Using these final values, the growth, development, and physiological behavior of cv. Hass avocado seedlings were simulated in controlled chambers for each cluster. Finally, as a means to mitigate the negative effects of temperature increases and precipitation reductions on these seedlings, nanoparticles of calcium phosphate were synthesized and applied via foliar and soil application after to standardized dosage (Fig. 1).

2.2. Determination of homogeneous climatic zones in avocado cv. Hass producing areas in Colombia

In order not to analyze climate variability in all precipitation and temperature weather stations in the study region (Fig. 2), and with the aim of reducing the evaluation area in zones when the climatic variability is homogeneous for the avocado-producing areas in Colombia we implemented a spatial clustering analysis based on climatological normal data (Figs. 1 and 2). Our approach was based on the premise that by grouping climate variability into homogeneous zones using multivariate analysis methods such as clustering,

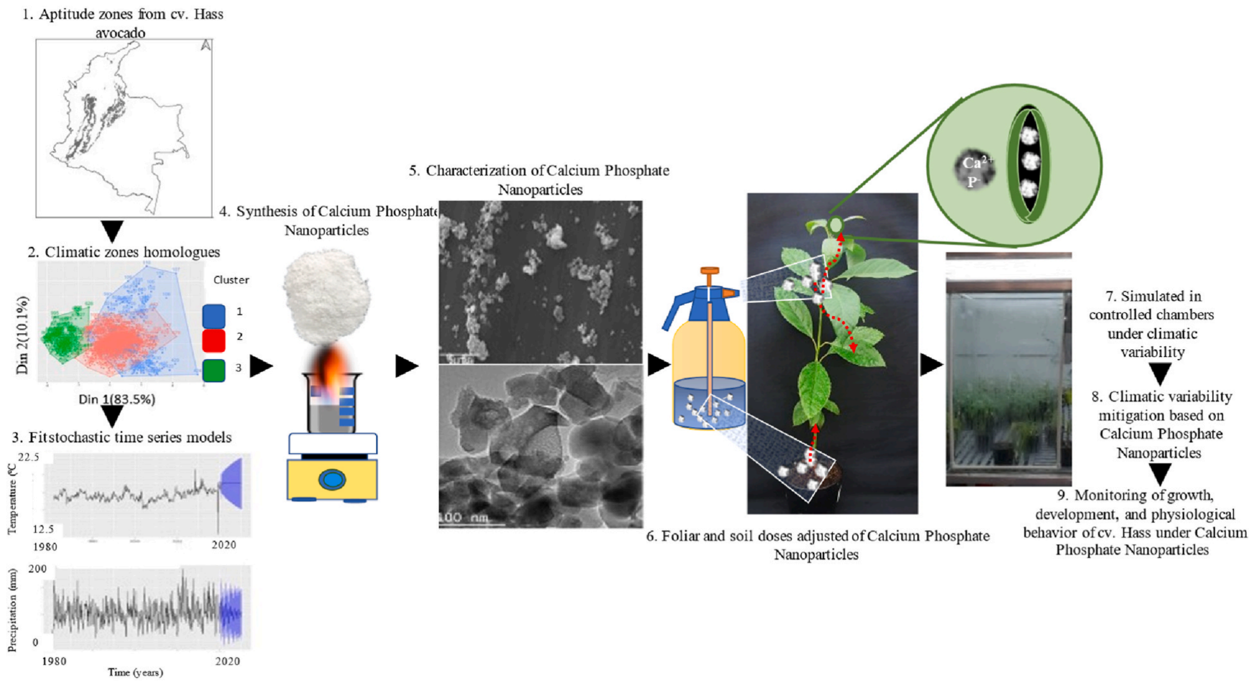

Fig. 1. Graphic scheme of the methodological approach.

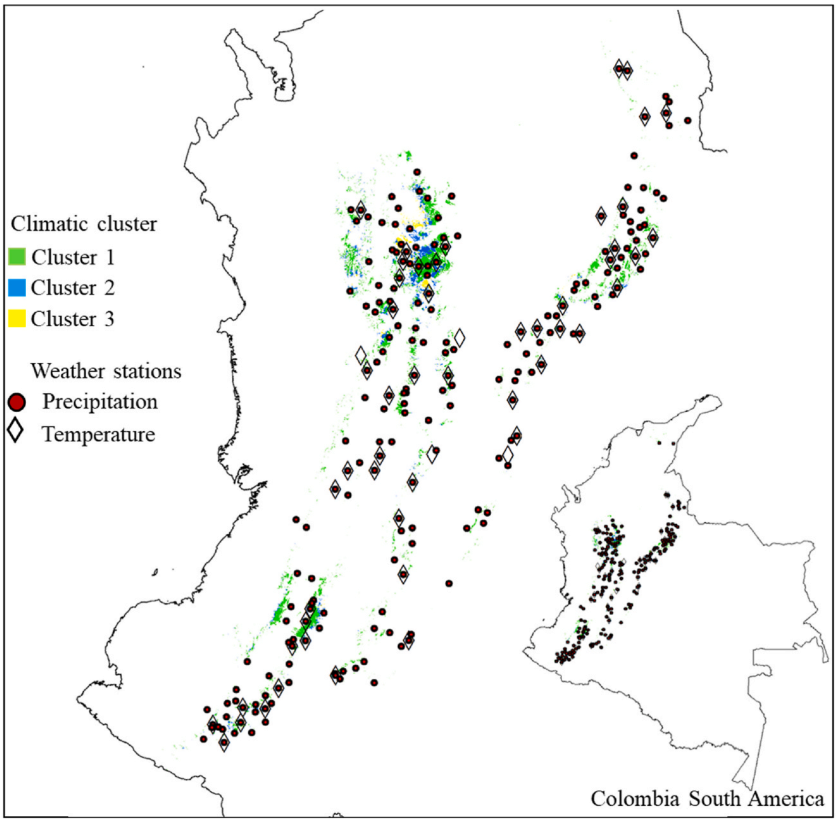

Fig. 2. Grouping of the climate variability based on K-mean method in cv. Hass avocado-producing areas in Colombia. Cluster 1: 1600–2200 m, total area: 65%. Cluster 2: 2201–2400 m, total area: 25%. Cluster 3: >2401 m, total area: 10%. The gap statistic and the lowest value of the error rule function E (675) were obtained with three clusters.

regions can be categorized into areas where climatic conditions exhibit similar behavior [55,56]. This facilitates the identification and characterization of climatic phenomena of importance at regional scales [55,56]. The homogeneous climatic zones from avocado in Colombia were made to the geographical distribution at a high spatial resolution (250 m) where this crop is planted [7]. The spatial variables used to this analysis were monthly temperature, precipitation, reference evapotranspiration using the Hargreaves formula [57], and the Martonne index [58]. Additionally, a digital elevation map with high spatial resolution (~30 m) was used to determine the elevation, along with the solar radiation, wind speed and vapor pressure. All variables described before were used spatially distributed at a resolution of ~1 km using the worldclim 2.1. API (Application Programming Interfaces) [59]. Later, the Pearson or Spearman correlation coefficient (CC) was determined for the linear dependence of the above variables, depending on the origin of the variable, eliminating those that were autocorrelated (CC $\geq$ 0.8).

The clustering analysis was implemented based on the spatial K-means method. K-means algorithm is a partitioning technique for clustering, and the most widely used such technique is spatial clustering. This is based on taking the mean value of each cluster centroid as the heuristic information [60]. The spatial K-means method was optimized based on a multi-step process: (i) standardization of variables based on the Z score methods [61] using the clusterSim library [62]; (ii) the optimal number of clusters estimated with the gap statistic [63] using the stat libraries [64]; (iii) select K-clustering centroids according to the Nearest-Neighbor rule [65], compute the mean value of each cluster and make it the new clustering centroid [66], and (iv) the assessment the best combination of parameters using the error rule function E [60]. All of these functions were implemented in the free software R [67].

2.3. *Modeling and simulation of temperature and precipitation in avocado cv. Hass producing areas in Colombia based on times series analysis*

For the clusters generated in the previous steps, the historical climatic database was built. Pluviometric and weather stations belonging to the Instituto de Hidrología, Meteorología y Estudios Ambientales (Ideam) (http://dhime.ideam.gov.co/atencionciudadano/) were selected in Colombian avocado cv. Hass production areas (Fig. 2A). We assume that the areas of influence of the buffer zone to temperature and precipitation of the weather stations were 5 and 10 km respectively (Fig. 2A). Subsequently, Pandas, NumPy, and Matplotlib libraries in the Python free software, using Colab work environment, were used for the data management of times series. The work sequence consisted of: (i) selection of stations with consistent values for the historical series from

1981 to 2020; (ii) initial visualization of the data; (iii) data cleaning and elimination of anomalous values, and (iv) calculation of the average, minimum and maximum monthly temperatures, along with monthly and annual accumulated precipitation.

For each cluster generated (Fig. 2), the data of average temperature and monthly accumulated precipitation for the 1981–2020 time series were selected. Then, the variations, trends, and forecasts associated with climatic variability were determined using stochastic time series models (STSM). The STSM approach is a widely used method to ascertain the behavior and make forecasts of climatic variables. It has practical uses in making decisions, risk management, operation of hydrological systems, quantification of uncertainty as a result of climatic variability, among others [68,69].

The time series data were adjusted to an autoregressive integrated moving average model (ARIMA) with the parameters (p,d,q) for the modeling and forecasting, where p denotes the number of autoregressive values; q denotes the number of moving average values and d is the order of differencing [68,69]. In the subsequent analysis we found that the analyzed series had trends (stochastic and deterministic), identified using the Dickey Fuller test and fitted to simple linear regression models with respect to time, violating the principles of this model [70]. Therefore, we used the *auto-arima* function of the forecast package [70] implemented in the free software R to model and find the parameters that best fit the time series of precipitation and temperature in each cluster. This algorithm automatically selects the ARIMA model that best fits the data based on the lowest AIC (Akaique information criterion) and RMSE (root-mean-square error) values. Additionally, and based on the Dickey Fuller test, we found that the series had a seasonality every 12 months. Given this, we decided not to differentiate it, and opted to force the *autoarima* function to make adjustments with $d = 0$ and $D = 0$ parameters to local and stational behavior of this series, thus fitting it to a to a model of the stationary autoregressive integrated of moving averages (SARIMA) type with the parameters (p,d,q) (P,D,Q)n, with d and $D = 0$ (stationary autoregressive of moving averages (SARMA)).

The forecast of the future behavior (expected future changes) of these two variables were adjusted to SARIMA or SARMA models by means of the normal distribution for errors (parametric bootstrap) and using the resampled errors (ordinary bootstrap), allowing a robust and applicable forecast [70]. This study followed the recommendation not to make forecasts beyond six periods for the correct use of the tool and was limited to a five-year projection (2021–2025). The changes in precipitation and temperature were obtained for the climatic clusters of the producing areas based on the deterministic trends obtained as the mean slope of the regression curve fitted to the time series for temperature and precipitation (Fig. 2A, B and C).

2.4. Synthesis and characterization of calcium phosphate nanoparticles

Calcium phosphates nanopowder was obtained using a solution combustion method previously reported [71]. Calcium nitrate tetrahydrate $Ca(NO_3)_2{*}4H_2O$ (Merck- CaN) and glycine $C_2H_5NO_2$ (Merck) were used as oxidant (O) and fuel (F) respectively, and diammonium hydrogen phosphate $(NH_4)_2HPO_4$) (Alfa Aesar-DHP) was used as a phosphate precursor. The ratio of Ca/P used was 1.5, which promotes the formation of a mixture of calcium phosphates corresponding to tricalcium phosphate (β−TCP) and hydroxyapatite (HAP) when combustion synthesis is employed [71,72]. First, to prepare 5 g of calcium phosphates, 4.25 g of DHP and 11.42 g of CaN were mixed in distilled water under constant stirring, until the formation of a white precipitate that was subsequently dissolved with 10 ml of nitric acid (HNO_3). Later 4.03 g of glycine was added, maintaining an oxidizer-to-fuel ratio $O/F = 1$ [73]. The final solution was maintained at approximately 80 °C under stirring until a gel appeared. Then, the temperature of the gel was increased to 200–250 °C until gel ignition. After that, the powders were recovered and characterized with X ray diffraction in a D8 Advance Eco Bruker model diffractometer from Bruker (Karlsruhe, Germany). In addition, the morphology of sample particles was observed with a scanning electron microscope (SEM, EVO MA10Carl Zeiss microscope) and transmission electron microscope (Tecnai F20 Super Twin TMP de FE).

2.5. Origin of seedlings and location of experiments for in vivo tests

The evaluation of the avocado seedlings was carried out at the Facultad Ciencias Agrarias, Universidad Nacional de Colombia, sede Medellin, using a growth chamber with temperature, radiation, and relative humidity automated panel control (Sanyo, Versatile Environmental TestChamber, model MLR- 352H). The greenhouse experiments and controlled conditions were carried out between July and December 2021.

The avocado seedlings were obtained from seeds of an adult clonal tree of the cv. Hass with visually confirmed health. These seeds underwent disinfestation and acceleration of germination [74]. Later, when these seedlings presented five fully expanded leaves, they were transplanted into plastic pots with 2 kg of soil on a wet basis. The soil was an and isol from an avocado-producing region with low concentrations of calcium and phosphorus in the soil (supplementary information 1). Until the beginning of the treatments, the plants were kept under net-house conditions (at 1,600 m altitude with a mean range temperature, relative humidity and solar radiation of 10–22 °C, 70–90% and 1,900–7,000 w m^{-2} respectively) at 50% of the maximum soil moisture retention capacity, and irrigated with a Hoagland-type nutrient solution [74].

2.6. Determination of the dose and method of application of calcium phosphate nanoparticles in avocado seedlings

First, given the lack of specific information associated with nanoparticles in avocado plants, the dose and optimal form of application of the calcium phosphate nanoparticles were determined based on previously reported doses in others plant species [42,75]. Three doses and two application methods were tested (foliar and soil): (i) D1: 15 mg l^{-1} (foliar) or g l^{-1} (edaphic); (ii) D2: 30 mg l^{-1} (foliar) or g l^{-1} (edaphic), and (iii) D3: 45 mg l^{-1} (foliar) or g l^{-1} (edaphic). The edaphic application (p:p) was carried out by direct addition to the soil in the form of a drench (dilution in 200 ml of sterile water) near the root zone (0.5 cm), distributed equidistantly from the stem. The foliar spray (p:v) was applied using manual equipment (10342.1 Pa) and hollow cone spray nozzles. A volume per plant of 200 ml in sterile distilled water was used. For both applications, the dispersion of the nanoparticles was carried out using an ultrasonic homogenizer (Hielscher UP400S).

In this previous phase the treatments evaluated were: T1: control (without application of nanoparticles and nutrient solution with P and Ca^{2+}); T2: D1-Foliar; T3: D2-Foliar; T4: D3-Foliar; T5: D1-Edaphic; T6: D2-Edaphic and T7: D3-Edaphic. A nutrient solution that maintained 50% of the maximum moisture retention capacity was used. Hoagland solutions both with and free of P and Ca^{+2} were used for control and the treatments where the nanoparticles were not used, respectively.

At the end of the experiment (120 days after the start of the experiment (das)), the dry biomass of the root and aerial part was evaluated, subjecting the samples to drying in an oven at 60 °C (Binder chamber®) until the mass stabilized. Additionally, the leaf area (cm^2) was determined using a LI-Cor® LI 3000a leaf area meter. Meanwhile, the foliar concentrations of P and Ca^{2+} were determined by means of non-destructive samples using the blue molybdate method [76] and tissue drying in an oven with a heating ramp of 2 °C every minute until reaching 550 °C, followed by acid digestion with 96% sulfuric acid and quantification with atomic absorption.

A completely randomized design was used, with five seedlings as the experiment unit, seven repetitions per treatment and two replicates over time. Data from the two replicates over time were used in the same analysis and time was not incorporated as a variance factor. Homocedasticity and normality of the data ($P < 0.05$) were analyzed using the Levene and Kolmogorov-Smirnov criteria, respectively. Subsequently, based on the fulfillment of the assumptions of the model the data were subjected to analysis of variance and post hoc analyses via comparison of means using Tukey's test ($P < 0.05$). This analysis was implemented using function in the free software R.

2.7. Stress reduction associated with climatic variability using calcium phosphate nanoparticles

This assay sought to evaluate whether calcium phosphate nanoparticles synthesized under conditions described before at doses of 30 mg l^{-1} via foliar application can reduce stress in avocado seedlings subjected to simulations of climate variability (Fig. 2). For this assay, automated growth chambers (described before) were used, and the simulation corresponded to the temperature and precipitation resulting from the forecast using the best SARIMA model for the three climatic clusters of the avocado-producing areas in Colombia (Fig. 2).

The seedlings were subjected to different scenarios of climate and water supply depending on the increase in temperature and reductions in precipitation (Fig. 2). Thus, the amount of water added to each pot was the result of subtracting the forecast variation from the monthly average of each cluster and taking into account the current moisture dynamics and maximum retention capacity of the soil over time, for which a set of soil moisture sensors calibrated for and isols from avocado-producing regions was used [77]. On the other hand, at the minimum, average and maximum temperatures according to the climatological normal in each cluster, the simulated value was increased (Fig. 2). The relative humidity and radiation conditions were used as the average of each cluster obtained from the worldclim free database [59].

Based on the previously mentioned information six treatments were designed. T1: control1- (without application of nanoparticles) + simulation-CC1 (+1.45 °C and −7.5 of average precipitation); T2: nanoparticles (30 mg l^{-1} via foliar application) + simulation-CC1; T3: control2- + simulation-CC2 (+1.28 °C and −5.2 of average precipitation); T4: nanoparticles (30 mg l^{-1} via foliar application) + simulation-CC2; T5: control3- + simulation-CC3 (+1.1 °C and −6.5 average precipitation); and T6: nanoparticles (30 mg l^{-1} via foliar application) + simulation-CC3. Hoagland nutrient solution with P and C^{2+} was used as nutrient solution for irrigation additions in control- and Hoagland nutrient solution without P and C^{2+} for treatments with nanoparticles.

The response variables were dry biomass of root and aerial part and leaf area (cm^2) determined at 120 days. Additionally, physiological variables associated with the stress response were evaluated: net photosynthesis (A), stomatal conductance (gs) and transpiration (E), quantified in a TPS-2 portable infrared gas analyzer (Portable PhotosynThesis System). The efficient use of extrinsic water (UEA)= (A/E) was determined from net photosynthesis and transpiration. These variables were determined in the morning hours (9–10 a.m.) and on the same leaves (third developed leaf, from the apical bud). Each quantification was repeated five times on the same tissue in each experiment unit for a period of 120 days, every 20 days (T1 = 10 days; T2 = 30 days; T3 = 60 days; $T4_=$ 90 and T5: 120 days). The specific condition of the variables measured with the equipment were: a external halogen lamp (LCi -ADC Bioscience, UK) with a constant irradiation of 1,320 μmol photons m^{-2} s^{-1}, PAR.

The experimental design experiment units, and the statistics analysis of the dry biomass variables (roots and aerial part) and leaf area, were carried out according to the experiment described in the section on dose and application of the nanoparticles. On the other hand, the physiological behavior variables were evaluated over time and on the same individuals. These variables were analyzed using a two-factor analysis of variance with repeated measurements over time, when the plants were considered as the within-subjects factor. Our analysis guaranteed the following assumptions: (i) normality (Shapiro-Wilks test); (ii) remove outliers using the box plot; (iii) sphericity to assume equality of variances using the Mauchly test. Based on the fulfillment of the assumptions and the fact that the factor presented significant differences ($P < 0.05$), we carried out a post hoc multiple comparisons analysis using Tukey's test. The analyses were performed using functions from the WSRS2 library [78], implemented in the free software R.

3. Results

3.1. Climatic characterization and variation in temperature and precipitation in avocado cv. Hass producing areas in Colombia

The multivariate analysis using the K-means method grouped the climatic variability of the cv. Hass avocado producing areas in Colombia into three spatial clusters (Figs. 2 and 3), explaining more than 93% of the data variation. In each cluster, a basic characterization was achieved based on the environmental variables with the greatest weight within the first three components. These three spatial clusters were obtained based on the gap statistic, centroids values statistically similar according to the Nearest-Neighbor rule,

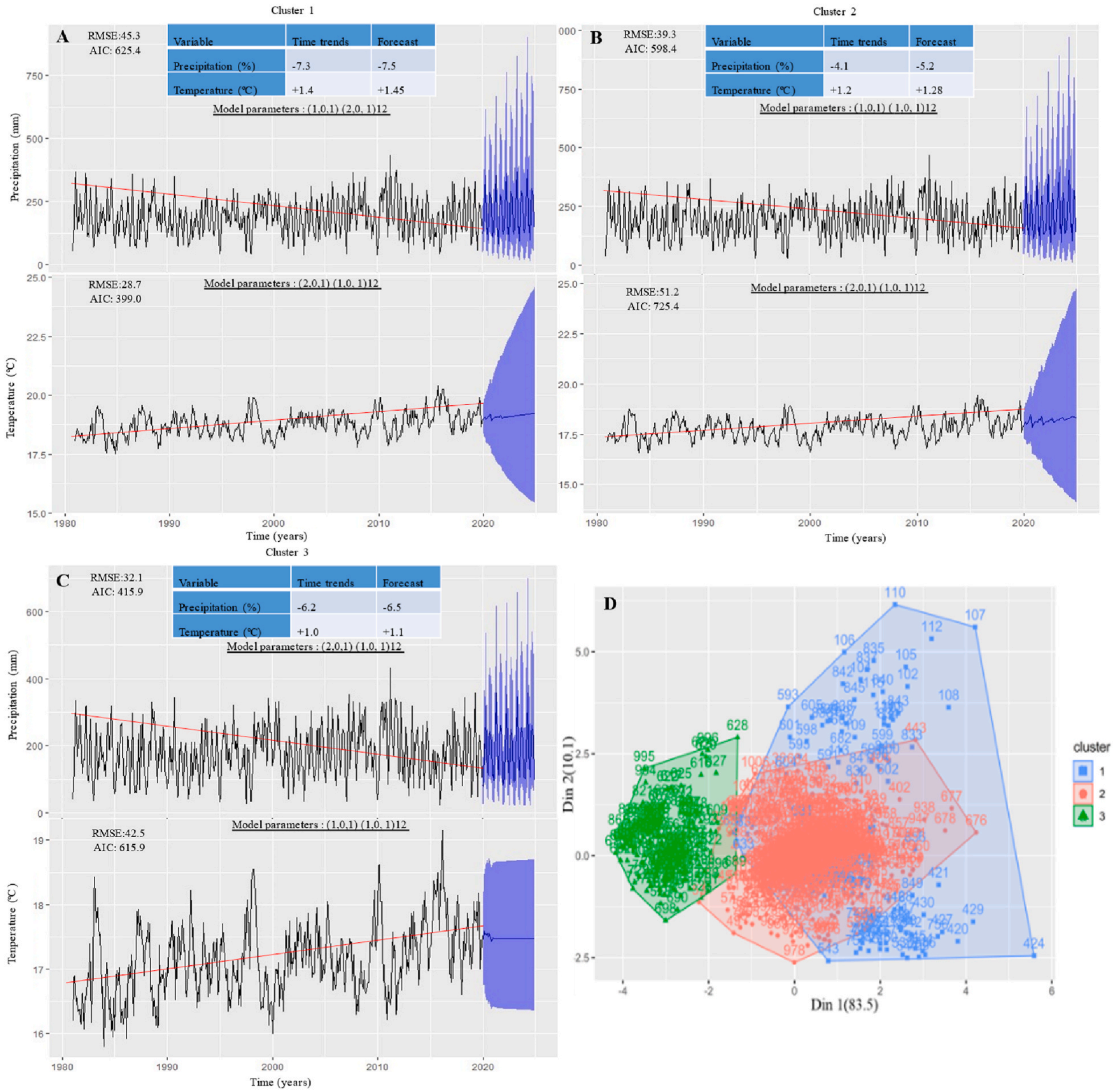

Fig. 3. Modeling and simulation of temperature and precipitation associated with climatic clusters in cv. Hass avocado-producing areas in Colombia using time series analysis stochastic methods. A, B and C: average temperature and monthly accumulated precipitation for the 1981–2020 fit using a stationary autoregressive integrated of moving averages (SARIMA) with the local and stational parameters (p,d,q) (P,D,Q)n for the modeling and forecasting. p denotes the number of autoregressive values; q denotes the number of moving average values and d is the order of differencing. RMSE: root-mean-square error. AIC: Akaike information criterion. Blue line and area mean forecast and forecast confidence interval. Red line means fit of the regression model to identify the deterministic trend of series. D: Graphical representation of the cluster analysis and assumed variation.

and lowest error rule function E (Figs. 2 and 3). Clusters 1, 2 and 3 presented the following physical and climatic characteristics: elevation between 1600 and 2200; 2201–2400 and > 2401 m; average temperatures in the range of 17.5–21, 16–19.5 and 14-19.5 °C; average monthly accumulated precipitation between 40 and 200, 30–150 and 35–180 mm; average annual solar radiation of 1750, 1700 and 1640 kWh m^{-2} $year^{-1}$; and sub-humid, humid, and humid Martonne aridity index, respectively.

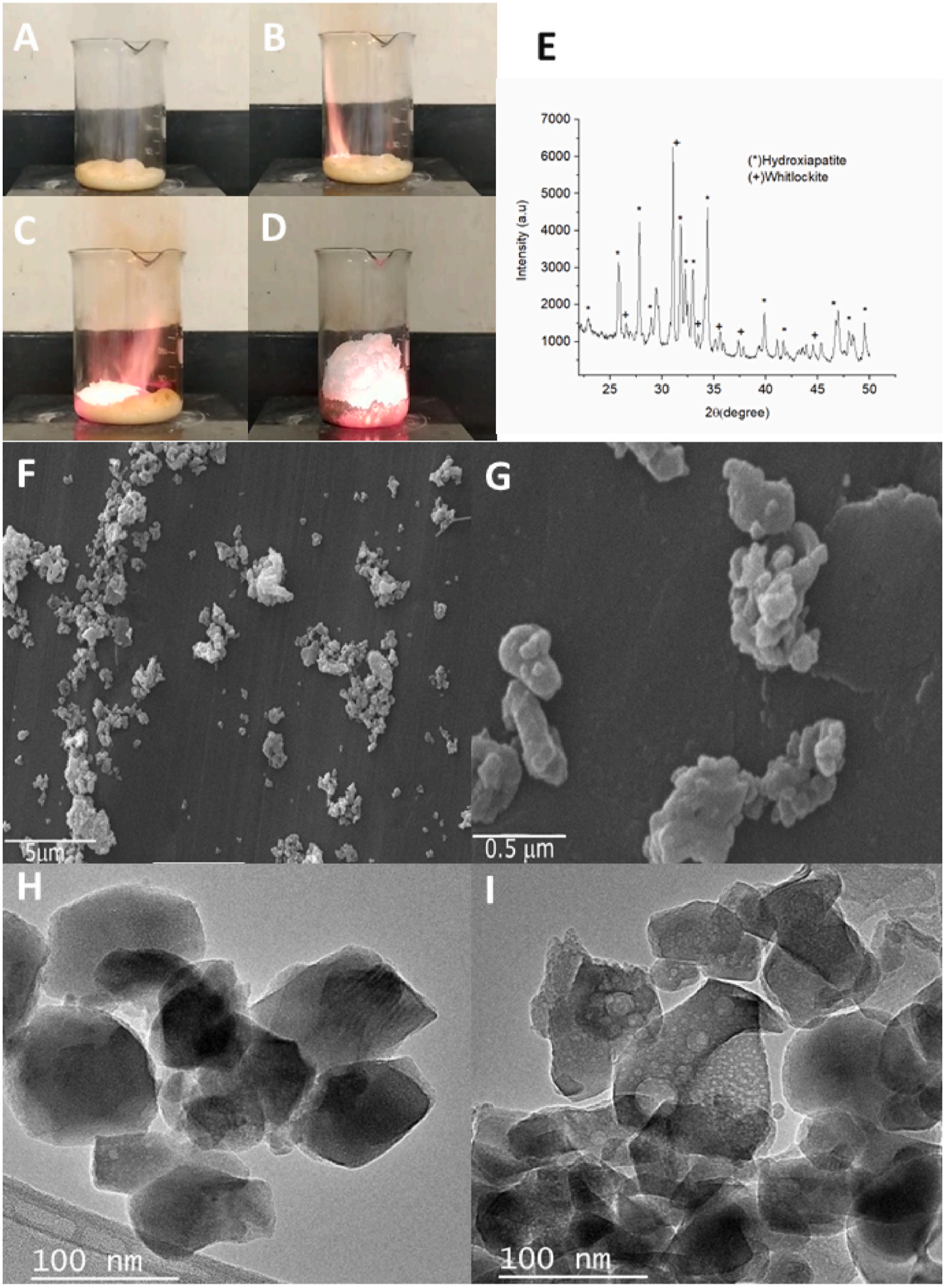

Fig. 4. Steps of the process of solution combustion method for the obtaining of Calcium phosphates nanoparticles. A-D: Photographs of combustion solution process; E: X-ray diffractogram of calcium phosphates; F-G: Scanning electron microscope of the powder, H–I) Transmission electron microscope of the powder.

The temporal analysis of the historical series of temperature and precipitation data for each cluster were fit to more than 3,500 models, where the best fit to the data was obtained with SARIMA modes (p,d,q) (P,D,Q)n, with four parameters associated with local and stationary effect and not integrated (d and D = 0). The specific configuration for each series of data from temperature and precipitation in the three clusters are reported in Fig. 3. Model selection was based on a balance between significance (*P value* < 0.05), less complexity with low AIC values and good prediction capacity when selecting the set of models with the lowest RMSE (Fig. 3 A, B, and C).

Based on the slope of the linear regression model curve associated with the deterministic trend of the series in the three clusters, the climate variability from the historical series was detected. We identified changes in the precipitation (reduction) and temperature (increase) for clusters 1, 2 and 3, at −7.3, −4.1 and −6.2% and 1.4, 1.2 and 1 °C, respectively (Fig. 3 A,B, and C). The forecast for the period 2021–2025 confirmed the trends for increases in temperature and reductions in precipitation obtained in the historical data series (Fig. 3 A,B, and C). Additionally, a stochastic trend was found every 12 months according to the Dickey Fuller test, indicating that there are variations every year, possibly associated with intra-annual variation (Fig. 3 A, B, and C).

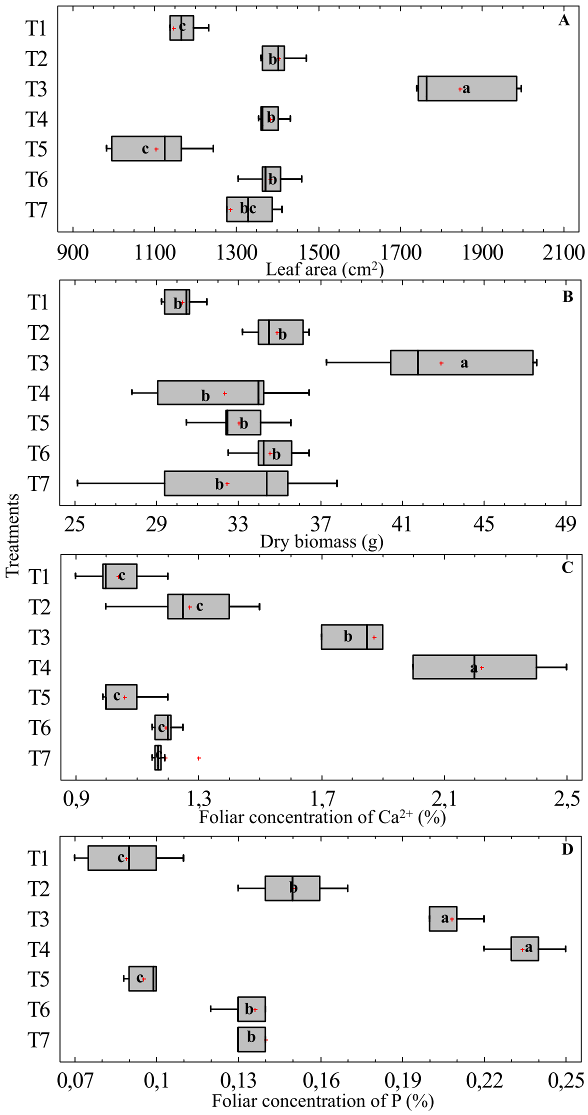

Fig. 5. Effects of dose and application of calcium phosphate nanoparticles in cv. Hass avocado seedlings on variables of growth, development, and foliar concentration of Ca^{+2}- and P^{-}. A-B: Biometric parameters. C-D: Concentration of nutrients Ca^{2+} and P. Error bars represent box and whisker plot. Letters represented the Tukey mean separation test. No overlapping of the error bars and equal letters indicates significant differences (*P*>*0.05*). D1: 15 mg l-1 (foliar) or g l-1 (edaphic); (ii) D2: 30 mg l-1 (foliar) or g l-1 (edaphic), and (iii) D3: 45 mg l-1 (foliar) or g l-1 (edaphic). T2: D1-Foliar; T3: D2-Foliar; T4: D3-Foliar; T5: D1-Edaphic; T6: D2-Edaphic and T7: D3-Edaphic.

3.2. Synthesis and characterization of calcium phosphate nanoparticles

Fig. 4A–D shows the combustion process to obtain calcium phosphates in a one-step stage. The reaction began with the formation of a gel until ignition with the presence of flame, passing through self-propagation until the final formation of phosphates (Fig. 4 D). The white color of the powders indicated phase formation. X-ray diffraction confirmed the presence of calcium phosphates (Fig. 4 E). The diffraction peaks associated with hydroxyapatite and tricalcium phosphates for Phase B were identified. It is important to highlight the high crystallinity of the materials obtained (Fig. 4 E).

The morphological results of the scanning electron microscopy (Fig. 4 F, G, H, and I) identified aggregate calcium phosphate structures with sizes smaller than 1 μm and composed of smaller particles, which indicated the presence of nanoparticles because of the reaction mechanics of the synthesis. The presence of nanoparticles was then corroborated. It was possible to identify particles around 100 nm, with a considerable presence of porosity associated with the high release of gases produced by combustion (Fig. 4 F, G, H, and I).

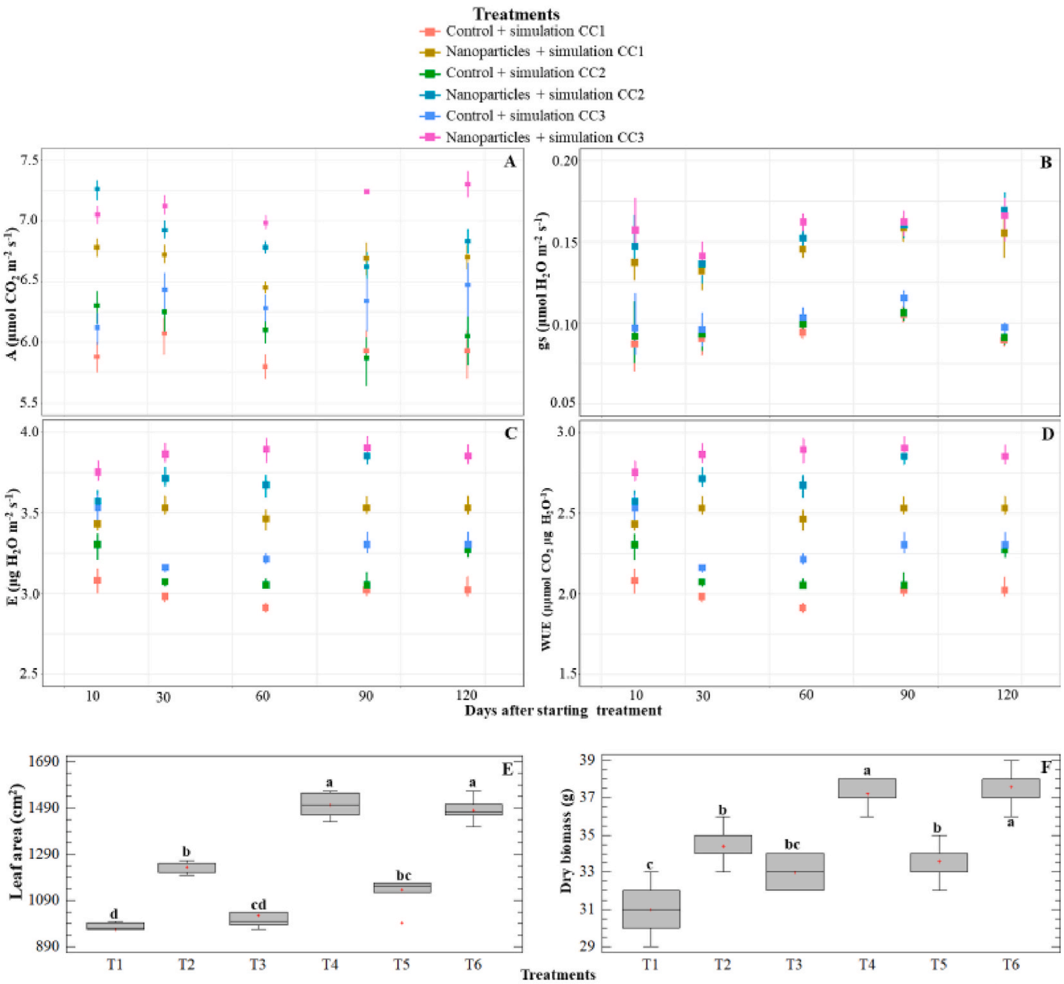

Fig. 6. Physiological and developmental monitoring of avocado seedlings subjected to stress due to temperature and water deficit under foliar application of calcium phosphate nanoparticles. CCn: represented the simulation of variation of temperature and precipitation associated with climatic cluster 1, 2 and 3 based on Figs. 1 and 2. A: Net photosynthesis. B: stomatal conductance. C: transpiration. D: water use efficiency. The data were analyzed through a two-factor analysis of variance with repeated measurements over time and post hoc multiple comparisons using Tukey's test. E and F: represent biometrics variables associated with growth and development variables. Error bars represent box and whisker plot. Letters represented the Tukey mean separation test. No overlapping of the error bars of equal letters indicates significant differences ($P>0.05$). T1: control1- (without application of nanoparticles) + simulation-CC1 (+1.45 °C and −7.5 of average precipitation); T2: nanoparticles (30 mg l-l via foliar application) + simulation-CC1; T3: control2- + simulation-CC2 (+1.28 °C and −5.2 of average precipitation); T4: nanoparticles (30 mg l-l via foliar application) + simulation-CC2; T5: control3- + simulation-CC3 (+1.1 °C and −6.5 average precipitation); and T6: nanoparticles (30 mg l-l via foliar application) + simulation-CC3.

3.3. Determination of the dose and method of application of calcium phosphate nanoparticles in avocado seedlings

For the dose and application method, the statistical tests showed significant differences ($P < 0.05$) for the treatments in the variables of growth and development (leaf area and dry biomass) and leaf concentration of P and Ca^{2+} (Fig. 5 A and B). For all variables, there was a high variation, presenting biases towards the lower and upper quartiles values in all the treatments (Fig. 5 A, B, C and D).

The leaf area was grouped into three classes ($P < 0.05$). The group with the lowest value was associated with the control and doses 1 (D1) with the edaphic method to nanoparticle application (Fig. 5 A). The group with intermediate values was grouped to treatments T2 (D1-Foliar), T4 (D3-Foliar), T6 (D2-Edaphic) and T7 (D3-Edaphic). On the other hand, higher leaf area values were seen in treatment T3 (dose 2 and foliar application) (Fig. 5 A). For biomass, there were two groups where, as with the previous variable, T3 presented higher values, while the other treatments (T2, T4, T5, T6 and T7) did not differ from the control (T1) (Fig. 5 B).

In the concentration of Ca^{2+}, the Tukey test was able to detect three groups (Fig. 5C). Dosage three, with a foliar application (T4), had the highest value, followed by treatment T3, while for treatments T2, T5, T6 and T7 it was not possible to identify differences with respect to control (T1) (Fig. 5C). For foliar P concentration, the statistical test gave rise to treatments T3 and T4, which presented the highest values, followed by T2, T6 and T7, while T5 and the control presented the lowest values without differences between them (Fig. 5 D).

3.4. Stress reduction in avocado seedlings associated with climatic variability using calcium phosphate nanoparticles

The analyses of the repeated measurements over time for the physiological variables, and variance analysis to growth and development variables showed significant differences ($P < 0.05$) (Fig. 6). In this sense, the results suggested two sources of variation when avocado seedlings were subjected to stress climatic condition: (i) climate cluster simulation and (ii) the effect of nanoparticles application (Fig. 6).

The simulation and response of the cv. Hass avocado seedlings under the three climatic variation scenarios associated with each cluster presented a differential behavior. Cluster 1 (greater reduction in precipitation and a higher increase in temperature) (Figs. 2 and 3) presented a greater negative effect on the physiological response and development of the plants. This stress situation gave rise to reductions of 15, 9, 12, 14, 25 and 20.5% in net photosynthesis, stomatal conductance, transpiration, efficient use of water, leaf area and biomass, respectively, with respect to cluster 3 (less variation in temperature and precipitation (Fig. 6). On the other hand, the response in cluster 2 (intermediate variation) presented an intermediate behavior when compared to clusters 1 and 2 (Fig. 6).

The effect of nanoparticles in each of the simulated scenarios (clusters 1, 2 and 3) showed that, for physiological response variables such as net photosynthesis, stomatal conductance, transpiration, and efficient use of water, an increase in the performance of cv. Hass avocado seedlings was achieved in the ranges between 10 and 30.7, 5.2–12.7, 15.5–45 and 7.5–24.8%, respectively, with respect to untreated seedlings (Fig. 6 A, B, C and D). The greatest reduction ($P < 0.05$) in the stress condition occurred in cluster 3, followed by clusters 2 and 1. Likewise, temporal variation was identified (Fig. 6). In the biometric variables, the physiological responses were corroborated, where the addition of nanoparticles enabled the increase in the leaf area and the dry biomass, with high variation within the treatments (Fig. 6 E and F).

4. Discussion

4.1. Changes in temperature and precipitation in Colombian producing areas of cv. Hass avocado under climate variability scenarios

This study indicated how climate variability has led to changes in precipitation (reduction) and temperature (increases) in avocado-producing areas in Colombia, with greater variation in those areas located at lower altitudes (Clusters 1 and 2) (Fig. 2). Climate variability phenomena, although influenced by regional factors governing the behavior of precipitation and temperature, such as ENSO and ITCZ, exhibit specific spatial and temporal patterns at the local level [17,21]. This pattern was identified and characterized in the present study, where it was found that clusters 1 and 2 exhibit greater climate vulnerability. These changes can alter the regimes of rains, droughts, floods, and evapotranspiration, among others [79], with a direct effect on the water needs of the avocado crop and its physiological, phenological and productive response [30]. This situation could have a negative environmental impact due to the possible use of water resources to supply water deficits in crops; with the question of high-water consumption currently being the subject of debate [80,81].

Based on these results and under tropical conditions, it is necessary to rethink the optimal areas for planting this fruit tree given the threat presented by climate variability to this fruit tree. In this regard, our approach of climatically homogeneous zones allowed us to identify specific climatic variations. This method provides a regional-scale approximation with a significant impact on the design of climate risk management policies for this crop, serving as a foundation for developing adaptation and mitigation strategies.

Variations in precipitation and temperature differentially affect the physiological, phenological and productive behavior of avocados [11,82]. Avocado production systems are highly vulnerable to increases in precipitation under ENSO-La Niña-type phenomena, which can alter the microbial dynamics in the soil, having impact on health, root development and productivity [7]. Meanwhile, increments in temperatures associated with ENSO-El Niño, can drastically reduce the yield and yield component [14]. The adaptation of plants to conditions of water scarcity or excess does not occur in short periods, resulting in a high impact on production [83].

Avocado is a plastic species in its response to temperature variation, given its wide distribution ranges, especially the Hass variety which can adapt to a wide variety of climatic conditions [84,85]. Despite this, the optimum is a range of diurnal and nocturnal temperatures of 21–25 and 14–18 °C, respectively [84,85], indicating that the producing areas located in cluster 1 could have conditions that restrict production. High temperatures cause heat stress, which causes damage to plant metabolism and development, but this is a complex phenomenon that involves the duration of stress, growth rate, and maximum temperature reached [86,87].

4.2. Responses of avocado seedlings cv. Hass to variations in temperature and precipitation associated with climate variability and adaptation strategies based on nanotechnology

Under simulated conditions, stress factors were reproduced in avocado seedlings associated with increases in temperature and decreases in precipitation in a differential way based on natural variation and forecasts for different producing regions in the tropical zone of Colombia. These responses were evident when changing the values of the physiological parameters. When plants are under abiotic stress such as drought and heat, the response is open the stomata to try to thermoregulate, but if the stress continue the response is stomata closure, which affects stomatal conductance and transpiration, among factors [88,89]. Similarly, stomatal closure causes a photosynthetic imbalance and therefore increases the production of reactive oxygen species (ROS) [90]. This induces a lower amount of CO_2, which affects net photosynthesis and dry matter production [91], giving rise to reduced growth as a visible sign of the stress event [88,89]. High temperatures disrupt the integrity of membranes, causing lipid peroxidation and compromising their functionality [88]. Regarding, heat stress can increase the photorespiration, as a mechanism by which plants try to minimize the harmful effects of excessive heat and oxidative stress [89], but the increased photorespiration leads to the consumption of energy and resources, reducing photosynthetic efficiency and overall plant productivity [92].

Recommendations for the search for elements that improve the behavior of cultivated plant species to phenomena of variability and climate change include tools that facilitate adaptation and mitigation [14,86,93]. Innovative strategies including the use of nanomaterials such as calcium phosphate nanoparticles are a tool with high potential for managing conditions of reduced precipitation and/or increases in temperature that lead to water and heat stress.

As shown in this research, the NPS concentration of 30 mg l^{-1} (T3) applied by foliar route achieved a reduction in water stress and resistance increment to heat compared to control. This may be related to the availability of nutrients and the speed with which they are incorporated by the plant [39]. In foliar application the NPs can enter the leaves through the stomata or cuticles. The cuticle and to a lesser extent stomatal pathway are the main route for plant foliar uptake of NPs. The length and width of stomata are usually at the scale of 11–13 μm in avocado and the nanoparticles with a size lower than this value can be transported through apoplastic and symplastic routes [39,94,95]. The smallest nanoparticles (between 10 and 50 nm) can be adsorbed through nanosized pores, and their transport favored through the adjacent cell's cytoplasm (symplastic route). Thus, median NPs (between 50 and 200 nm) are translocated between the cells (apoplastic route) [39,94,95]. On the other hand, larger nanoparticles can be adsorbed through their degradation mechanisms, and they could release different ions for their incorporation into the plant [37]. In the case of NPs applied to soil, these can penetrate plant roots through osmotic pressure, capillary forces, or by passing directly through the root epidermal cells [39,96]. Subsequently, these can be translocated to aerial tissues after bioaccumulation in cellular or subcellular organelles in the roots [97].

As demonstrated in this work, the effect of NPs on avocado seedlings is influenced by the dose and the form of application. NPs present interaction with plants at cellular and subcellular level, inducing morphological, physiological, biochemical and genetic changes [38,39,98]. These interactions may be positive or negative, depending on the NPs type, reactivity, size, concentration and bioaccumulation in the cells [94,96,97,99,100]. In maize plants, the use of calcium phosphate nanoparticles has been shown to improve development and physiological behavior [75]. It is speculated that the mechanisms that explain the better adaptation of plants to extreme events under the influence of nanomaterials are related to enzymatic activity and genetic regulation [101,102]. Therefore, foliar application could be the most efficient method of nutrient supply for the plant [37,100], but it is necessary to identify the correct dose.

Common commercial fertilizers, such as monoammonium phosphate (MAP), diammonium phosphate (DAP) or triple superphosphate (TSP), are water – soluble salt phosphates that are readily dissolved in soil solution [49]. However, these soluble phosphates are also highly mobile in soil, and most enter water surface bodies through runoff and natural infiltration [48]. Suspension of calcium phosphate nanoparticles can be used to add calcium and phosphorous nutrition to the plant with conventional methods such as spraying or irrigation. Also, calcium phosphate nanoparticles have a much weaker interaction with soil components than PO_4^{3-}, HPO_4^{2-}, or Ca^{2+} ions, Therefore, much more phosphorus and Ca ions remain in the culture medium for plants [49].

The advantage of using NPs rather than other fertilizers, especially through foliar application, is that direct interaction with soil systems is avoided, eliminating potential ecological risks [37,100]. This may be due to the characteristics of andosol soil, which has a large presence of allophane clays that could complex the ions, reducing their mobility and incorporation into the plant through the roots [103]. In this sense, engineered nanoparticles of Ag and Cu could increase Pi retention in soil size fractions, which can decrease soil fertility [104].

The beneficial effects of the application of calcium (Ca^{+2}) have been demonstrated under different conditions of stress, giving rise to increases in physiological variables such as photosynthesis, stomatal conductance, gas exchange, and grain yield [105,106]. The possible mechanisms by which Ca^{+2} improves drought tolerance are due to the fact that this element intervenes in stress signaling pathways [107,108]. Likewise, calcium can act as a secondary messenger [107,108] and increase the integrity of the membranes [107,108], leading to an improvement in the functionality of the photosynthetic apparatus through gas exchange [107,108]. The application of Ca^{+2} can act as a physiological treatment to increase drought tolerance in plants, among other benefits [105,106]. However, is important to determinate dosage levels to avoid any potential adverse effects. Excessive intracellular calcium concentrations can activate endonuclease enzymes, leading to DNA chain breaks and alterations in genes associated with mitochondrial cell death regulation [49]. In our study, a concentration of 30 mg L^{-1} of calcium phosphate seems to be well-tolerated by avocado plants, aligning with findings from other authors who have reported the safety of various calcium phosphate concentrations when administered through foliar application [51].

Meanwhile, phosphorus is an essential element in mineral nutrition that fulfills multiple roles, notable in photosynthesis, respiration, energy generation, nucleic acid biosynthesis, and as an integral component of plant structures, amount others [109,110]. Despite its importance, scarcity of this mineral limits crop yield in more than 40% of the world's arable land [109]. The low availability of phosphorus (P) under tropical conditions can be attributed to multiple factors. One factor is that the soil itself may be deficient in phosphorus, leading to limited P availability for plants. Additionally, when phosphorus fertilizers in commercially available forms are applied, they may become adsorbed by the soil particles, reducing their accessibility to plants [103]. Another factor is that phosphorus is highly mobile in soil, and a significant portion of it can be carried away by runoff and natural infiltration, eventually entering surface water bodies [103]. Therefore, the low availability of phosphorus in tropical conditions can be a result of both soil deficiencies and the loss of phosphorus through runoff and infiltration, leading to reduced accessibility of phosphorus for plant growth [103]. Faced with cost of P fertilizers, its poor availability in the soil, the low assimilation and efficiency in plant uptake, and the losses through leaching, calcium phosphate nanoparticles are a viable solution for phosphoric fertilization.

One of the most important drawbacks in the application of nanoparticles in the agricultural field is their difficulty of production [37,38]. This requires the implementation of mechanisms to obtain adequate performance for continuous production [49]. In this case, calcium phosphate nanoparticles were obtained by combustion in a solution method in one single step. When the ignition temperature of the system was reached (Fig. 2B), the heat released made it possible to obtain the calcium phosphates in a single stage (Fig. 2D).

Previous studies have reported that theoretical combustion temperatures can be around 800–900 °C. These temperatures were sufficient for the formation of different biphasic calcium phosphates with a high degree of crystallinity [71]. Solution combustion is a suitable route of synthesis that provides methods for the high production of calcium phosphate nanoparticles for potential use of nano-CaP in precision agriculture for the controlled delivery of new fertilizer formulations.

In summary, the use of calcium phosphate nanoparticles could increase agricultural productivity and resistance to abiotic stresses in avocado plants. Their application may help to decrease the use of common fertilizers by the smart delivery of active ingredients such as Ca^{2+} and phosphorus ions and to increase nutrient uptake. At the same time, they could decrease fertilizer losses from volatilization, leaching, runoff, and consumed energy during production. Furthermore, the use of calcium phosphate nanoparticles may decrease the costs of agricultural production and environmental issues, as well as increasing nutrient use quality. Application of different types of nano-fertilizers has a major impact on crop yields, the protection of natural resources and the reduction of fertilizer cost for crop production.

5. Conclusions

We found that climate variability associated with variations in temperature (increases) and precipitation has differentially affected avocado-producing areas in Colombia highly related to homogeneous climatic zones identified by special cluster analysis using the K-means method. The simulation of climatic variables based on the climatic clusters generated a negative effect on the growth, development, and physiological behavior of avocado seedlings that was directly proportional to the magnitude of the increase in temperature and the reduction in precipitation. In addition, our results indicated that nanoparticles of calcium phosphate are a promising candidate for the development of fertilizers to provide nutrients needed for the avocado plants and could minimize adverse effects on physiology, growth, and development associated with climatic variability, especially water stress, high temperature, and water consultation. Indeed, further investigations are warranted to elucidate the intricate mechanisms underlying the interaction between calcium phosphate nanostructures and plants. These studies should also strive to identify the lower and upper threshold doses that might induce cytotoxicity. By comprehending the dose-response relationship, researchers can establish precise application rates and guidelines for the agricultural utilization of calcium phosphate nanostructures. Assessing the cytotoxic effects across a range of doses will significantly contribute to ensuring the overall safety and efficacy of these nanostructures in plant-based applications.

Author contribution statement

Joaquín Guillermo Ramírez-Gil and Alex A. Lopera, C. García: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data and Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18658.

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