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Morpho-physiological response of *Ochroma pyramidale* produced in nurseries by biopot technology to N, P, K fertilisation using an optimal custom design

Respuesta morfofisiológica de *Ochroma pyramidale* producida en viveros mediante tecnología biopot a la fertilización con N, P, K utilizando un diseño óptimo personalizado

Resposta morfofisiológica da pirâmide *Ochroma pyramidale* cultivada em viveiros empregando a tecnologia de fertilização N, P, K com um projeto ideal feito sob medida

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ABSTRACT

Ochroma pyramidale is a fast-growing species with high commercial value. The largest volumes of its wood are obtained from commercial plantations; however, there are very few studies of its production in nurseries. The objective of this study was analyse the effect of N, P, K concentration and fertilisation frequency on morpho-physiological response of *O. pyramidale* grown in biopots using response surface methodology. The experiment was set up using the response surface methodology for which 30 seedlings were used per experimental run. The experiment consisted of applying different concentrations of N, P, K and two fertilisation frequencies according to the optimal (Custom) design. The response variables were height increase, diameter increase and CO₂ assimilation. From the results obtained, we confirmed the positive effect of N, P and K fertilisation on the variables under study. From the experimental data, a second-order quadratic polynomial model was found that allows the prediction of the morpho-physiological response of the species. The frequency of fertilisation during the time interval studied was not significant.

Keywords: fertilisation; response surface methodology; *Ochroma pyramidale*.

RESUMEN

Ochroma pyramidale es una especie de rápido crecimiento y alto valor comercial. Los mayores volúmenes de su madera se obtienen de plantaciones comerciales; sin embargo, existen muy pocos estudios sobre su producción en vivero. El objetivo de este estudio fue analizar el efecto de la concentración de N, P, K y la frecuencia de fertilización sobre la respuesta morfofisiológica de *O. pyramidale* cultivada en tubetes utilizando la metodología de superficie de respuesta. El experimento se estableció utilizando la metodología de superficie de respuesta para lo cual se utilizaron 30 plántulas por corrida experimental. El experimento consistió en aplicar diferentes concentraciones de N, P, K y dos frecuencias de fertilización según el diseño óptimo (personalizado). Las variables de respuesta fueron el aumento de altura, el aumento de diámetro y la asimilación de CO₂. A partir de los resultados obtenidos, se confirmó el efecto positivo de la fertilización con N, P y K sobre las variables en estudio. A partir de los datos experimentales se encontró un modelo polinómico cuadrático de segundo orden que permite predecir la respuesta morfofisiológica de la especie. La frecuencia de fertilización durante el intervalo de tiempo estudiado no fue significativa.

Palabras clave: Fertilización; Metodología de superficie de respuesta; *Ochroma pyramidale*.

RESUMO

A *Ochroma pyramidale* é uma espécie de crescimento rápido com alto valor comercial. Os maiores volumes de sua madeira são obtidos de plantações comerciais; no entanto, há muito poucos estudos sobre sua produção em berçário. O objetivo deste estudo foi analisar o efeito da concentração de N, P, K e frequência de fertilização na resposta morfofisiológica da pirâmide de *O. pyramidale* cultivada em biopots usando a metodologia de superfície de resposta. O experimento foi implementado utilizando a metodologia da superfície de resposta, para a qual foram utilizadas 30 mudas por série



experimental. A experiência consistiu na aplicação de diferentes concentrações de N, P, K e duas frequências de fertilização de acordo com o projeto ideal (Personalizado). As variáveis de resposta foram aumento da altura, aumento do diâmetro e assimilação de CO₂. A partir dos resultados obtidos, o efeito positivo da fertilização N, P e K sobre as variáveis em estudo foi confirmado. A partir dos dados experimentais, foi encontrado um modelo polinomial quadrático de segunda ordem para prever a resposta morfofisiológica da espécie. A frequência da fertilização durante o intervalo de tempo estudado não foi significativa.

Palavras-chave: Fertilização; Metodologia de superfície de resposta; *Ochroma pyramidale*.

INTRODUCTION

Ochroma pyramidale (balsa) is a species native to the Americas, ranging from southern Mexico to Peru. It is a fast-growing species that reaches 20m in height and up to 75cm in diameter in 5-8 years. Given the physical and mechanical properties of its wood, it has been widely used for different purposes such as toys, handicrafts, interior veneer, insulation and pulp for paper (Borrega *et al.*, 2015). Therefore, balsa wood is in high demand in the international market, mainly in Europe, China and the United States. It is also used in restoration projects for degraded areas (Miyajima *et al.* 2018; Cañadas-López *et al.*, 2019).

In its natural habitat, the species occurs as individual trees in forest clearings in tropical rainforests and in mixed groups with other species. It rarely grows in dense stands (Fletcher 1951). The high dispersal of balsa over large areas of forest makes extraction difficult and costly. For this reason, most of the commercially used timber is from plantations, particularly from Ecuador (Borrega *et al.*, 2015).

Despite the demand for the species, there has not been a great deal of much research into the management and production of the species, so further studies are needed. In this regard, fertilisation in nurseries to obtain nourished seedlings that respond better to field conditions is a topic that remains a novelty. Many authors argue that fertilisation in containers at the nursery stage increases survival by improving the plants' physiological state during the establishment phase through increased root growth (Luis *et al.*, 2009; Trubat *et al.*, 2010). Therefore, the objective of this research was to analyse *Ochroma pyramidale* produced in nurseries using biopot technology with the aim of analysing the effect of N, P, K concentration and fertilisation frequency on morpho-physiological response of *O. pyramidale* grown in biopots using response surface methodology (RSM).

RSM has been used in several areas of research due to the advantages it offers, as it considerably reduces the experimental runs. In addition, it allows one to find a predictive model of the independent variables from the study factors.



MATERIALS AND METHODS

Location of the experiment

The experiment was carried out in the nursery located in the central campus of the Universidad Estatal Amazónica, Puyo, Ecuador (1° 27' 59.8" S; 77° 59' 51.6" W) at an average maximum and minimum temperature inside the nursery of 28 °C and 17 °C (Davis Vantage Pro 2 weather station), respectively.

Biological material and experimental conditions

Seeds were collected from *O. pyramidale* trees in Arajuno parish, Pastaza, Ecuador (1° 13' 01.8" S; 77° 39' 08.5" W, 1° 15' 58.1" S; 77° 37' 31.4" W, 1° 16' 27.4" S; 77° 41' 20.3" W). Subsequently, in the laboratory, the seeds were selected according to their morphology and placed in a container with water to eliminate the empty seeds. They were disinfected with 0.5% sodium hypochlorite and soaked for 24 hours as a pre-germinative treatment. Next, they were sown directly in earthworm humus with a composition of 2 % nitrogen, 0.48% phosphorus and 1.13 % potassium, and a pH of 7.08. In addition, 10 % (v/v) of rice husk was added to encourage aeration. Seedling production was carried out in black biopots of 110 cm⁻³. Fertilisation was initiated when the seedlings reached three months of age (30 seedlings per experimental run). Three fertilizer were used as independent source of N, P and K. Nitrogen source was urea (46 % N), rock phosphate (28 % P) and potassium chloride (60 % K) respectively.

Morpho-physiological response

At fourth month from the cultivation was assessed the response to increase height (cm), diameter (mm) and CO₂ assimilation (μmol m⁻²s⁻¹). In case of photosynthetic assimilation rate (A) was measured with a portable integrated photosynthesis and chlorophyll fluorescence measurement system with fully programmable microclimate control (iFL/Cpro-SD, ADC BioScientific Ltd., Herts, UK). Determinations were performed between 8:00 h and 11:00 h on ten seedlings, as proposed by [Ávila-Lovera & Tezara \(2018\)](#) and [Ávila-Lovera et al., \(2019\)](#), on mature, intact leaves. The operating conditions were ambient CO₂ (~ 400 μmol mol⁻¹) assisted by a compressed CO₂ cylinder, 21 % O₂, Photosynthetic photon flux density 1000 μmol m⁻²s⁻¹ leaf temperature similar to ambient 25.4 ± 0.2°C and a vapour pressure deficit of 4.36 ± 0.4 kPa.

Optimal (custom) design

Using Design Expert software version 12.0 (serial number 9847-9696-7992-6750, Stat-Ease Inc., 1300 Godward Street North, Suite 6400 Minneapolis, USA), the optimal (custom) design (Table 1) was performed with N, P, K concentration as a numerical factor and fertilisation frequency as a categorical factor. The range of N, P, K concentrations (75-150 mg L⁻¹) in the experiment was selected according to the trials conducted by [Basave-Villalobos et al., \(2020\)](#). ANOVA was applied to analyse the influence of N, P, K concentration on height increase, diameter increase and CO₂ assimilation of *O. pyramidale* as independent variables (P < 0.05). The veracity of the model was determined by the coefficient of determination (R²) and significance (P) (Table 1).



Table 1. - Optimal (custom) design for the independent variables (N, P, K concentration and fertilisation frequency) and experimental and predicted results of height increase, diameter increase and CO₂ assimilation (CO₂ A.) of *Ochroma pyramidale*

Run	Concentration N, P, K (mg L ⁻¹)	Frequency	Height increase (cm)		Diameter increase (mm)		CO ₂ A. (μmol m ⁻² s ⁻¹)	
			Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	121.88	1	2.08	1.05	1.04	1.04	9.83	9.15
2	112.50	2	1.73	0.96	0.98	0.98	8.12	8.94
3	93.75	2	1.54	0.95	0.88	0.88	8.01	7.52
4	140.01	1	3.51	1.20	1.23	1.23	11.61	11.89
5	131.25	2	2.92	1.14	1.14	1.14	10.17	11.24
6	85.88	1	1.46	0.80	0.84	0.84	5.33	6.10
7	75.00	1	1.04	0.83	0.82	0.82	5.48	5.80
8	75.00	2	1.50	0.85	0.85	0.85	7.97	6.96
9	104.63	1	2.01	0.94	0.92	0.92	8.71	7.29
10	150.00	2	4.58	1.33	1.35	1.35	14.64	14.4
11	112.50	2	1.87	0.94	0.98	0.98	9.04	8.94
12	140.01	1	4.00	1.20	1.23	1.23	11.45	11.89
13	140.01	1	4.16	1.29	1.23	1.23	11.61	11.89
14	75.00	2	1.09	0.83	0.85	0.85	6.21	6.96
15	150.00	2	4.56	1.39	1.35	1.35	15.19	14.40

RESULTS

In order to analyse the effect of N, P, K concentration and fertilisation frequency on height increase, diameter increase and CO₂ assimilation of *O. pyramidale* grown in biopots, fourteen experimental runs were carried out, starting from the optimal (custom) design, which allows the highest increase in the variables considered. Table 1 shows the experimental results and those predicted for the construction of the model. Increases in height were between 1.32 and 4.53cm and in diameter were between 0.8 and 1.29mm respectively. Regarding assimilation rates, the species presented minimum values of 5.31mol m⁻²s⁻¹ and maximum values of 15.21mol m⁻²s⁻¹. Of the models predetermined by the design (Table 2), it was found that = the best fit for all variables was the quadratic model with an R²= 0.9257 (increase in height), R²= 0.9413 (increase in diameter) and 0.9361 (CO₂ assimilation). These results indicate that 92.5 %, 94.1 % and 93.6 % of the total variation of the variables was determined by the concentration of N, P, K applied (Table 2).



Table 2. - Summary of the polynomial models analysed by the Design Expert Software on the effect of N, P, K concentration and fertilisation frequency on height increase, diameter increase and CO₂ assimilation of *Ochroma pyramidale* grown in biopots

Height increase (cm)	Sequential P-value	Lack of Fit P-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.0313	0.8455	0.7996	
2FI	0.954	0.0236	0.8316	0.7676	
Quadratic	0.0003	0.3604	0.9537	0.9257	Suggested
Cubic	0.2909	0.3789	0.9575	0.9103	
Quartic	0.3796	0.2927	0.959	0.7069	
Fifth	0.2927		0.9614		Aliased
Diameter increase (mm)	Sequential P-value	Lack of Fit P-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.0705	0.8958	0.862	
2FI	0.9455	0.0541	0.8864	0.8264	
Quadratic	0.0008	0.4648	0.9611	0.9413	Suggested
Cubic	0.8594	0.2849	0.9532	0.8808	
Quartic	0.4235	0.1716	0.9531	0.5099	
Fifth	0.1716		0.9627		Aliased
Assimilation (µmol m ⁻² s ⁻¹)	Sequential P-value	Lack of Fit P-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.0693	0.8636	0.8157	
2FI	0.972	0.0531	0.8512	0.7788	
Quadratic	0.0163	0.1504	0.9106	0.8554	Suggested
Cubic	0.0881	0.2841	0.9391	0.7907	
Quartic	0.2752	0.2692	0.9472	0.5753	
Fifth	0.2692		0.9516		Aliased

Figure 1 (A, C and E) shows the predicted and experimental values 120 days after germination for height increase, diameter increase and CO₂ assimilation of *O. pyramidale* grown in biopots. The distribution of points corroborated the ability of the model to cover the whole experimental range. The R² and adjusted R² values of the regression lines are close to one, indicating a very good correspondence between the experimental and predicted values of the model on the experimental data. Figure 1 (B, D and F) shows that the experimental data met the assumption of normal distribution (Figure 1).



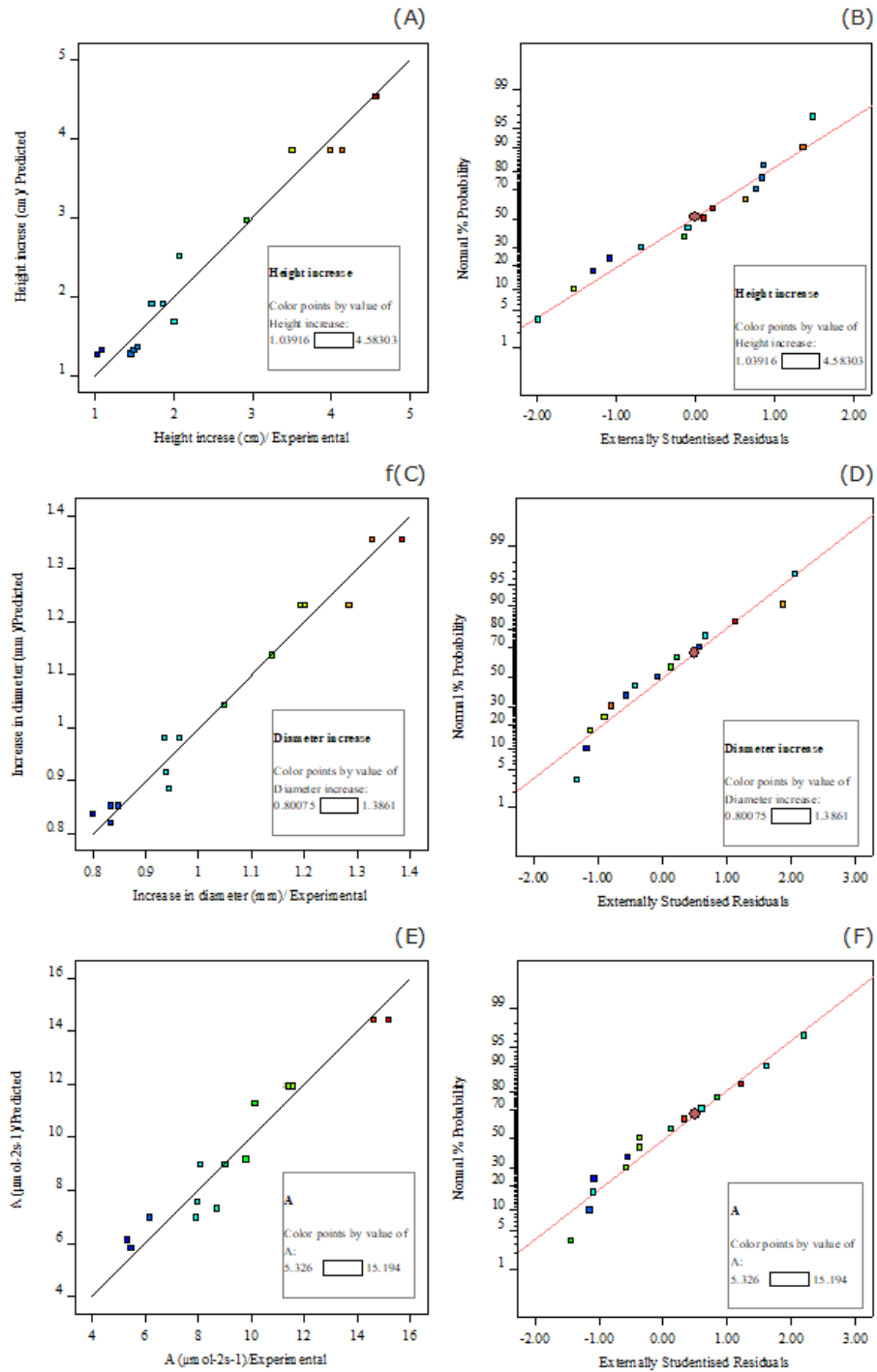


Figure 1. - Experimental versus predicted values for optimal (custom) design: (A) increase in height; (C) increase in diameter; (E) CO₂ assimilation; (B, D and F) Normal distribution of experimental data



Also, according to the results in Table 3, it was confirmed that the concentration of N, P, K was a significant factor ($P < 0.05$) in the morpho-physiological response of the species for the three response variables, whilst the frequency of fertilisation for the period evaluated was not significant ($P > 0.05$).

Table 3. - ANOVA for Response Surface Quadratic model

Height increase (cm)	Sum of Squares	df	Mean Square	F-value	P-value	
Quadratic	0.523	4	0.1307	87.51	< 0.0001	significant
A-Concentration	0.5	1	0.4995	334.58	< 0.0001	
B-Frequency	0.001	1	0.0006	0.3939	0.5443	
AB	0.001	1	0.0007	0.4586	0.5136	
A²	0.033	1	0.033	22.13	0.0008	
Residual	0.015	10	0.0015			
Lack of Fit	0.008	5	0.0016	1.09	0.4648	not significant
Pure Error	0.007	5	0.0014			
Corrected Total	0.538	14				
Diameter increase (mm)	Sum of Squares	df	Mean Square	F-value	P-value	
Quadratic	22.524	4	5.631	73.073	2.31E-07	significant
A-Concentration	20.945	1	20.945	271.795	1.41E-08	
B-Frequency	0.04	1	0.04	0.519	4.88E-01	
AB	0.045	1	0.045	0.581	4.64E-01	
A²	2.313	1	2.313	30.009	2.70E-04	
Residual	0.771	10	0.077			
Lack of Fit	0.45	5	0.09	1.400	3.60E-01	not significant
Pure Error	0.321	5	0.064			
Cor Total	23.295	14				
Assimilation ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Sum of Squares	df	Mean Square	F-value	P-value	
Quadratic	115.83	4	28.96	36.64	< 0.0001	significant
A-Concentration	108.83	1	108.83	137.69	< 0.0001	
B-Frequency	2.95	1	2.95	3.73	0.0823	
AB	0.119	1	0.119	0.1506	0.7061	
A²	6.56	1	6.56	8.3	0.0163	
Residual	7.9	10	0.7904			



Lack of Fit	5.76	5	1.15	2.69	0.1504	not significant
Pure Error	2.14	5	0.428			
Cor Total	123.73	14				

Based on the height increase, diameter increase, CO₂ assimilation of *O. pyramidale* and the desirability function, a second-order quadratic polynomial regression equation was established in terms of coded values (Eq. 1, Eq. 2 and Eq. 3 below). According to this model, the dependency relationship for each study variable and the applied N, P, K concentrations can be predicted. The graphical representations of the regression equations are shown in Figure 2 (B, C and D). The statistical significance of the regression equations concerning the polynomial model was tested using the F-test and ANOVA (Table 3) and (Figure 2). (Equation 1); (Equation 2) and (Equation 3).

$$\text{Height increase} = 1.95 + 1.69A - 0.0523B - 0.078AB + 1.03A^2 \quad (1)$$

$$\text{Diameter increase} = 0.9732 + 0.2606A - 0.0063B - 0.0096AB + 0.1230A^2 \quad (2)$$

$$\text{CO}_2 \text{ assimilation} = 8.49 + 3.85A - 0.4490B - 0.1272AB + 1.73A^2 \quad (3)$$



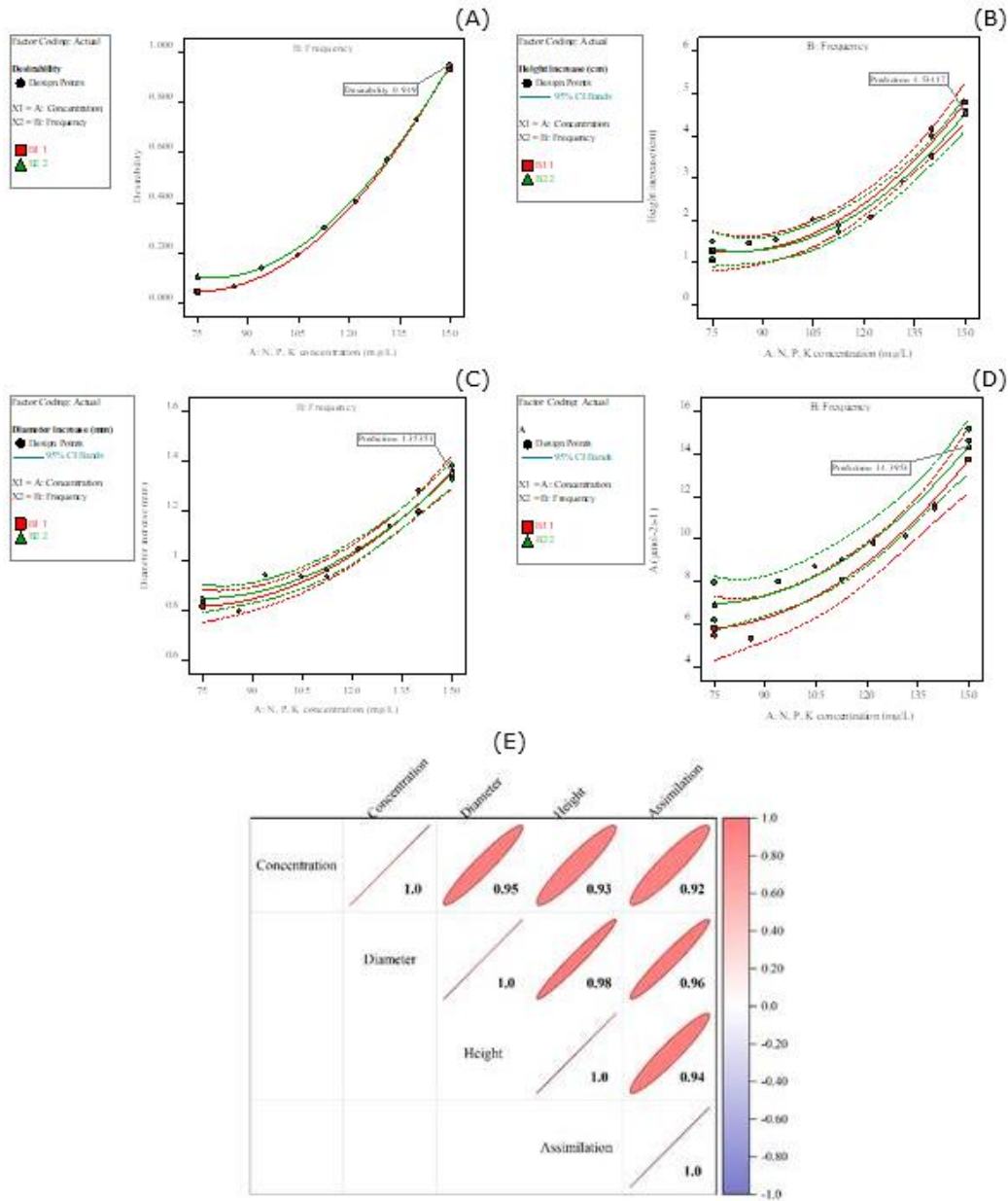


Figure 2. - Model graph for optimal (custom) design: (A) desirability; (B) height increase; (C) diameter increase; (D) CO₂ assimilation; (E) correlation between N, P, K concentration and response variables



DISCUSSION

Cultural fertilisation practices in the nursery are important in the cultivation of container-grown seedlings. These practices have a strong influence on the morphological development and nutrient levels of the seedlings (Villar-Salvador *et al.*, 2009). The results obtained in our investigation show that with higher concentrations of N, P and K, biomass production was favoured. This was reflected in the increases in height and diameter as a consequence of greater CO₂ assimilation, given the high correlation found between the variables (Figure 3E).

The positive effect of N, P, K fertilisation has been documented by various authors. For example, Hawkins *et al.*, (2005) reported that applying nutrients to *Tsuga heterophylla* (Raf.) Sarg. seedlings improved the growth. Other authors stated that nitrogen, phosphorus and potassium are the three primary macronutrients that favour photosynthetic rate, root growth and efficient water use, respectively (Fernández *et al.*, 2006). Oliet *et al.*, (2005) argued that cultural regimes at the nursery stage influence plant quality. They examined the influence of mineral nutrition at the nursery stage on *Acacia salicina* Lindl. that was planted on a degraded site in southeastern Spain. They found that survival was significantly higher for seedlings fertilised at high rates and that the initial benefits to field growth associated with nursery fertilisation diminished after four years. The survival of nourished seedlings was significantly higher than that of non-nourished seedlings after prolonged drought after the sixth year. The results suggest that the mineral nutrient status of nursery material (especially high P content) may positively affect the long-term establishment of *A. salicina* seedlings in semi-arid Mediterranean climates.

It has also been found that the fertilisation of *Quercus ilex* seedlings in nurseries influences morphophysiology and yield in the field (Andivia *et al.*, 2011). The fertilisation of *Pinus tabuliformis* Carr. in nurseries also indicated positive effects on seedling growth (Shi *et al.*, 2019). In an investigation of alternative substrates and fertiliser doses in the production of *Pinus cembroides* Zucc. in nurseries, the authors concluded that the use of a higher fertiliser dose, regardless of the substrates used, promoted higher plant growth responses (Madrid-Aispuro *et al.*, 2020). In another experiment to analyse the influence of mineral fertilisation in different growing media on the growth of *Inga edulis* Mart seedlings, the positive effect of fertilisation was also demonstrated (Mahmoud and Hussein 2021).

In a study on nutrient content and photosynthesis in young *O. pyramidale* plants carried out in the central Brazilian Amazon, it was found that with adequate foliar levels of nitrogen and phosphorus, the CO₂ assimilation rate was 13 μmol m⁻²s⁻¹ (Marenco *et al.*, 2001). The values found in our investigation for the highest dose of N, P, K used agree with those reported by these authors and corroborate the physiological response of the species to fertilisation.

CONCLUSIONS

Using the response surface methodology, a second-order quadratic polynomial relationship was found between the independent variables (concentration of N, P, K and frequency of fertilisation) and the dependent variables (height increase, diameter



increase and carbon dioxide assimilation). In the interval studied, the frequency of fertilisation was not significant, so the use of a single initial dose is better. The higher concentration of nutrients applied had an effect on the increase in height and diameter, as well as the higher rate of CO₂ assimilation.

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Conflict of interests:

The authors declare not to have any interest conflicts.

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The authors have participated in the writing of the work and analysis of the documents.



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