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Research Article

Effect of Pectimorf on the rooting ability, and morpho-physiological trials of national cocoa (*Theobroma cacao* L.) under different substrates

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Abstract

Cocoa is an economical cash crop that is formerly planted worldwide. Cuttings are a method of vegetative propagation suitable for maintaining desirable characteristics in cocoa trees. A greenhouse experiment was performed to evaluate the optimal concentrations of Pectimorf (0, 10, 50, and 100 mg L^{-1}) for rooting ability and seedling establishment as well as some physiological trials of 4 months EETP-800 national cocoa cuttings grown under two different substrates (S1: 80% soil + 20% sand and S2: 70% soil + 20% sand + 10% rice husk). The data showed that in most cases there are no significant differences in vegetative growth and root characteristics as well as gas exchange parameters between the two substrates. On the other hand, the application of Pectimorf concentration enhanced all tested traits compared to untreated plants. The most effective in this regard was 100 mg L^{-1}, that giving the highest value of all trials. As for the interaction effect, the result also shows that the application of Pectimorf concentration in special at 100 mg L^{-1} among two substrates had an additive effect on plant growth, gas exchange, and survival percentage compared to non-treated cuttings. The application of 100 mg L^{-1} Pectimorf with S2 substrate produced stronger seedlings with a higher survival percentage. This protocol can be used commercially for cocoa propagation commercially.

Keywords: bioregulator; cuttings; root length; photosynthesis

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Introduction

Cocoa (*Theobroma cacao* L., family Malvaceae) is an important global cash crop, and a compelling source of farmers' income (Zamora *et al*., 2022), with yields greater than 600 Kg ha^{-1} (ICCO, 2022). Consistent with the latest available statistics from the International Cocoa Organization, the total global production of cocoa beans in 2020-2021 was 5.2 million metric tons (ICCO, 2022). Cocoa is economically, socially, and environmentally important in Ecuador, it is currently the third largest non-oil product export in the country (García-Briones *et al*., 2021). It occupies 20% of the total national agricultural area, of that, about 50% of that area corresponds to National type cocoa, called "fine aroma" (INEC, 2018). The production of cocoa bars and chocolate, which are popular worldwide for their delectable flavor and nutritional value, needs cocoa beans as a necessary component (Barrientos, 2015). Additionally, there are a number of other non-food uses for cocoa beans in the manufacture of cosmetics and medications (Wickramasuriya and Dunwell, 2018).

Commercial cacao cultivations are started from seeds since this method of propagation is more convenient and affordable than others (Sodré and Gomes, 2019). However, there are some difficulties in maintaining the superior characteristics of selected cocoa trees when they are propagated from seed, primarily because of plant cross-pollination or allogamy, which prevents the appropriate agronomic characteristics of some chosen individuals from persisting over successive generations (Zamora *et al*., 2022). In order to produce clones plants that will keep the necessary characteristics, such as a high yield and resistance to pests and diseases, vegetative propagation is used in the cocoa industry (Suchithra, 2018). Several countries i.e., Ecuador, Brazil, Costa Rica, and Colombia have utilized vegetative propagation methods extensively and reported an improvement in their productivity (Reyes-Pérez *et al*., 2021a; Tovar *et al*., 2022). Superior cocoa cultivars have been multiplied and preserved by the use of clonal propagation methods including air layering and grafting, however, these processes are time and can lead to incompatibility issues between the scion and rootstock (Sodré and Gomes, 2019). Cutting plant propagation is a potential, practical, and quick technical alternative for plant growth while preserving the mother plant's genetic characteristics (Zamora *et al*., 2022). Cutting based asexual propagation depends on taking non-flowering branches with mature, healthy leaves and treating them with phytoregulators to promote root initiation and aerial part budding, ultimately producing a new plant that is genetically similar to the original (Enríquez, 2004). Wiesman and Jaenicke (2002), reported that several endogenous and exogenous aspects like hormonal balance, mineral and health status of cuttings, age of the cutting, and propagation setting have an impact on the success of this process.

Biostimulants (phytoregulators) have been reported to boost crop yields and quality, induce resistance and regulate growth and development processes (Acosta *et al*., 2018; Shahrajabian *et al*., 2021). Pectimorf is an inexpensive biostimulant and constitutes a feasible alternative for the substitution of plant hormones such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA) (Borges-García *et al*., 2015; Suárez-Guerra and Hernández-Espinosa, 2015). Pectimorf is composed of oligogalacturonides, which are obtained from citrus industry waste with a degree of polymerization between 9 and 16 molecules of galacturonic acid, it increases plant growth and development, as well as increases several crops' yield (Acosta *et al*., 2018; Reyes-Pérez *et al*., 2021a). Pectimorf has shown proficiency in promoting adventitious root development in different plant species (Falcon and Cabrera, 2007; Hernández *et al*., 2009; Kollarova *et al*., 2012; Lara *et al*., 2018). The best concentration of rooting-promoting substances, however, must be chosen in accordance with the species, genotype, and characteristics of the cuttings (Reyes-Pérez *et al*., 2021b). For cocoa, Ramirez *et al*. (2003) proved that cuttings belonging to diverse cultivars had dissimilar rooting reactions in response to Pectimorf concentration.

The main objective of the current study was to evaluate the effect of different concentrations of Pectimorf (0, 10, 50, and 100 mg L^{-1}) on the rooting efficiency and growth of National cocoa seedlings (EETP-

800) established in different substrates, hoping to establish an efficient protocol for propagation of cocoa cultivars.

Materials and Methods

Experimental location

The current research was conducted in a controlled greenhouse at the Faculty of Agricultural Sciences in the Mocache canton area, "La María" campus, Los Ríos province, which is located at a kilometer 7.5 of the Quevedo. The geographical location of the experimental site is 01° 06' 24" South latitude and 79° 29' 70" West longitude, at an altitude of 75 meters above sea level. The region experiences a humid tropical climate, with an average annual temperature of 24.8 °C, average rainfall of 2,252.5 mm year^{-1}, daylight of 894 hours year^{-1}, and relative humidity of 84%.

Plant material

The clone EETP-800, which was recently released by the National Institute for Agricultural Research (INIAP) was used. It is a cross between CCN 51 x EET-233 (Loor-Solórzano *et al*., 2019). This clone maintains the organoleptic properties of National type cocoa while producing more than CCN 51 (Jaimez *et al*., 2018).

Experimental design

A completely randomized block design with a factorial arrangement was employed: 4 concentrations of Pectimorf x 2 different substrates with 3 repetitions, considering 10 seedlings as the experimental unit, for a total of 240 plants. Factor 1: 0, 10, 50 and 100 mg L^{-1} Pectimorf (D_0; D_1; D_2; D_3, respectively) and factor 2: 80% orchard soil + 20% sand (S_1) and 70% orchard soil + 20% sand + 10% rice husk (S_2): obtaining a total of 8 treatments (S_1D_0, S_1D_1, S_1D_2, S_1D_3, S_2D_0, S_2D_1, S_2D_2, S_2D_3) made up of the interactions of the two factors (Table 1).

Table 1. Treatments used in the experiment

Treatments
Substrates
S_1: 80% orchard soil 'T' + 20% sand 'A'
S_2: 70%T + 20% A + 10% rice husk 'CA'
Pectimorf concentrations (mg L^{-1})
D_0: 0
D_1: 10
D_2: 50
D_3: 100
Interactions
S_1D_0: 80%T + 20% A + 0 mg L^{-1}
S_1D_1: 80%T + 20% A + 10 mg L^{-1}
S_1D_2: 80%T + 20% A + 50 mg L^{-1}
S_1D_3: 80%T + 20% A + 100 mg L^{-1}
S_2D_0: 70%T + 20% A + 10% CA + 0 mg L^{-1}
S_2D_1: 70%T + 20% A + 10% CA + 10 mg L^{-1}
S_2D_2: 70%T + 20% A + 10% CA + 50 mg L^{-1}
S_2D_3: 70%T + 20% A + 10% CA + 100 mg L^{-1}

Cuttings treatments

The chosen National cocoa cuttings (5-10 cm long, each cutting had 3-5 axillary buds, and leaflets were trimmed to 30% of their area) were disinfected with 1% sodium hypochlorite and then dipped in Pectimorf concentration with 10 cuttings of clone EETP-800. The cuttings were then planted in the appropriate substrate. Weekly irrigations were carried out with an atomizer for maintaining the appropriate level of substrates wetting.

Growth variables

At 60, 75, 90, and 120 days from treatment (DFT), plant height, stem diameter, and the number of leaves per plant were measured. Ten seedlings were randomly chosen for each treatment. At 120 DFT, each treatment's dry weight and root biomass were calculated and each seedling's adventitious roots were counted, its root length from the stake's neck to its terminal apex was measured

Gas exchange

With a portable infrared gas analyzer (CIRAS-II, PP Systems Inc., Amesbury, MA) at 120 DFT, the following parameters were measured for each treatment between 08.00 and 12.00 h net photosynthetic rate (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E). The fully expanded and healthy third upper leaf was used for all measurements. The following conditions were used: CO_2 concentration of 400 ±10 μmol mol^{-1}, leaf temperature of 28.0 ± 1 °C, gradient leaf-air vapor density (Δ_W) of 2.4 ± 0.5 KPa, and photosynthetic photon flux density (PFFD) of 1000 ± 50 μmol m^{-2} s^{-1} (light was provided by a light unit based on LEDs from the same manufacturer). The instantaneous water use efficiency (WUE) was estimated as: WUE = A/E.

Cocoa seedling survival

The survival percentage was determined at 120 DFT, taking into account the relationship between the total number of plants sown and the number of plants alive within each experimental unit.

Statistical analysis

STATISTICA v10 (StatSoft Inc., Tulsa, OK, USA) was used to perform the two-way analysis of variance (ANOVA), and Tukey's test at 95% probability was used to compare the means of the substrates, dose of Pectimorf, and interactions with a significance level of $p < 0.05$. Plots were made with Sigmaplot 11 (Systat Software, Inc., San Jose, CA).

Results

Plant height and stem diameter

Average seedlings heights ranged from 26.16 to 32.70 cm, with no significant differences between substrates at 60 and 75 DFT within two different substrates; however, significantly higher heights were observed in S_2 at 90 (31.32 cm) and 120 (32.70 cm) DAS (Table 2). No significant differences in stem diameter were observed between the substrates (Table 3). The concentration of 100 mg L^{-1} of Pectimorf caused a significant average increase of 12.8 and 19.4% in the plant height and stem diameter, respectively over untreated cuttings. No significant differences were observed in the interaction (substrate x Pectimorf) for these variables (Tables 2 and 3). However, in both substrates there was a positive effect of the Pectimorf treatment, the greatest plant height and stem diameters of the cocoa seedlings were observed in S_1D_3 and S_2D_3, that is, where the highest concentration of Pectimorf was applied. In each of the substrates, treatment D_0 (0 mg L^{-1}) showed the lowest plant height and diameter of the stem.

Table 2. Height and diameter of cocoa clone EETP-800 seedlings established in two types of substrate, subjected to different concentrations of Pectimorf

Treatments	Plant height (cm)			
	60 DFT	75 DFT	90 DFT	120 DFT
Substrates				
S_1: 80%T + 20% A	26.16 ± 0.36 a	28.13 ± 0.54 a	30.32 ± 0.53 a	31.55 ± 0.61 a
S_2: 70%T + 20% A + 10% CA	26.17 ± 0.19 a	28.59 ± 0.39 a	31.32 ± 0.36 b	32.70 ± 0.49 b
p (substrate)	*0.9840*	*0.2923*	*0.0259*	*0.0105*
Pectimorf concentrations (mg L^{-1})				
D_0: 0	25.69 ± 0.30 a	26.66 ± 0.32 a	28.90 ± 0.60 a	29.61 ± 0.58 a
D_1: 10	26.05 ± 0.46 a	27.81 ± 0.43 ab	30.75 ± 0.42 a	31.91 ± 0.51 b
D_2: 50	26.26 ± 0.47 a	28.85 ± 0.48 b	31.39 ± 0.36 ab	33.10 ± 0.42 ab
D_3: 100	26.67 ± 0.33 a	30.13 ± 0.16 c	32.23 ± 0.36 b	33.88 ± 0.28 b
p(Pectimorf)	*0.4555*	*0.0002*	*0.0002*	*0.0000*
Interactions				
S_1D_0	25.48 ± 0.53 a	26.42 ± 0.60 a	28.12 ± 0.79 a	29.00 ± 0.95 a
S_1D_1	25.75 ± 0.92 a	27.08 ± 0.82 ab	29.98 ± 0.35 bc	30.88 ± 0.27 abc
S_1D_2	26.48 ± 0.88 a	29.03 ± 1.05 c	31.18 ± 0.75 abc	32.55 ± 0.69 bcd
S_1D_3	26.95 ± 0.49 a	30.00 ± 0.28 c	31.98 ± 0.75 c	33.75 ± 0.60 d
S_2D_0	25.91 ± 0.31 a	26.89 ± 0.29 ab	29.68 ± 0.73 ab	30.21 ± 0.64 ab
S_2D_1	26.05 ± 0.33 a	28.55 ± 0.51 bc	31.52 ± 0.42 bc	32.94 ± 0.44 bcd
S_2D_2	26.34 ± 0.54 a	28.67 ± 0.15 bc	31.60 ± 0.22 bc	33.65 ± 0.36 cd
S_2D_3	26.39 ± 0.51 a	30.26 ± 0.18 c	32.47 ± 0.16 c	34.02 ± 0.07 d
p (substrates x Pectimorf)	*0.7042*	*0.5034*	*0.6246*	*0.4970*
Mean	26.17 ± 0.02	28.36 ± 0.33	30.82 ± 0.33	32.13 ± 0.40
Coefficient of variation (%)	3.99	3.64	3.30	3.06

* T: Orchard soil; A: Sand; CA: Rice Husk; PD: Pectimorf concentration. The level of significance (p) is shown in italics for each treatment: substrates, Pectimorf concentration and interaction (substrates x Pectimorf). Means with the same letter in each data group do not differ statistically according to Tukey's test (p>0.05).

Table 3. Diameter of cocoa clone EETP-800 seedlings established in two types of substrates, subjected to different concentrations of Pectimorf

Treatments	Stem diameter (mm)			
	60 DFT	75 DFT	90 DFT	120 DFT
Substrates				
S_1: 80%T + 20% A	5.26 ± 0.19 a	5.85 ± 0.11 a	6.15 ± 0.11 a	6.29 ± 0.12 a
S_2: 70%T + 20% A + 10% CA	5.33 ± 0.17 a	5.88 ± 0.15 a	6.19 ± 0.11 a	6.32 ± 0.14 a
p (substrate)	*0.4731*	*0.7581*	*0.5028*	*0.8525*
Pectimorf concentrations (mg L^{-1})				
D_0: 0	4.63 ± 0.11 a	5.48 ± 0.10 a	5.82 ± 0.06 a	5.93 ± 0.09 a
D_1: 10	5.18 ± 0.09 b	5.64 ± 0.04 ab	5.96 ± 0.04 ab	6.03 ± 0.11 ab
D_2: 50	5.21 ± 0.11 b	5.90 ± 0.12 b	6.20 ± 0.07 b	6.35 ± 0.10 b
D_3: 100	6.17 ± 0.10b	6.43 ± 0.10 c	6.72 ± 0.06 c	6.92 ± 0.04 c
p(Pectimorf)	*0.0000*	*0.0000*	*0.0000*	*0.0000*
Interactions				
S_1D_0	4.44 ± 0.11 a	5.52 ± 0.14 a	5.81 ± 0.1 a	5.89 ± 0.11 a
S_1D_1	5.14 ± 0.14 ab	5.77 ± 0.01 ab	5.92 ± 0.08 a	6.08 ± 0.08 a

S_1D_2	5.34 ± 0.21 b	5.91 ± 0.26 abc	6.22 ± 0.15 ab	6.42 ± 0.08 abc
S_1D_3	6.11 ± 0.23 c	6.31 ± 0.11 bc	6.66 ± 0.03 bc	6.87 ± 0.01 bc
S_2D_0	4.82 ± 0.12 ab	5.43 ± 0.17 a	5.82 ± 0.1 a	5.97 ± 0.17 a
S_2D_1	5.21 ± 0.15 b	5.53 ± 0.16 a	6.00 ± 0.01 a	5.97 ± 0.22 a
S_2D_2	5.08 ± 0.08 ab	5.89 ± 0.06 abc	6.18 ± 0.01 a	6.28 ± 0.20 ab
S_2D_3	6.22 ± 0.0 c	6.56 ± 0.14 c	6.77 ± 0.13 c	6.96 ± 0.09 c
p (substrates x Pectimorf)	*0.2296*	*0.4085*	*0.8129*	*0.7576*
Mean	5.30 ± 0.13	5.86 ± 0.09	6.17 ± 0.08	6.31 ± 0.09
Coefficient of variation (%)	4.77	4.33	2.51	3.75

* T: Orchard soil; A: Sand; CA: Rice Husk; PD: Pectimorf concentration. The level of significance (p) is shown in italics for each treatment: substrates, Pectimorf concentration and interaction (substrates x Pectimorf). Means with the same letter in each data group do not differ statistically according to Tukey's test (p>0.05).

Number of leaves per seedlings

The number of leaves per seedling varied from 2.73 to 6.53 with no significant differences between the two substrates at 60, 75, and 90 DFT; however, the highest number of leaves was observed in S2 at 120 DFT (Table 4). At 60, 75, 90, and 120 DFT, respectively, the concentration of Pectimorf at 100 mg L^{-1} (D_3) significantly increased the number of leaves by 30.3; 23.6; 18.20; and 45.4 percent in comparison to 0 mg L^{-1} (D_0). The interaction between the substrate and Pectimorf had no discernible effects on the number of leaves per seedling. However, the Pectimorf treatment had a good impact on both substrates; the largest number of leaves per plant was seen in S_1D_3 and S_2D_3, respectively, that is, where 100 mg L^{-1} of Pectimorf was applied. In each of the substrates, treatment D_0 (0 mg L^{-1}) showed the fewest number of leaves.

Table 4. Number of leaves per seedling of the cocoa clone EETP-800 established in two types of substrates in response to the application of different concentrations of Pectimorf

	Leaves number per seedlings			
	60 DFT	75 DFT	90 DFT	120 DFT
Substrates				
S_1: 80%T + 20% A	2.73 ± 0.09 a	3.27 ± 0.01 a	5.06 ± 0.01 a	6.20 ± 0.26 a
S_2: 70%T + 20% A + 10% CA	2.78 ± 0.16 a	3.39 ± 0.09 a	5.07 ± 0.19 a	6.50 ± 0.27 b
p(substrates)	*0.6352*	*0.0670*	*0.9174*	*0.0047*
Pectimorf concentrations (mg L^{-1})				
D_0: 0	2.56 ± 0.13 a	3.04 ± 0.08 a	4.67 ± 0.13 a	5.26 ± 0.11 a
D_1: 10	2.43 ± 0.13 a	3.12 ± 0.05 a	4.93 ± 0.05 ab	6.16 ± 0.10 b
D_2: 50	2.69 ± 0.06 a	3.41 ± 0.07b	5.13 ± 0.08 b	6.38 ± 0.11 b
D_3: 100	3.33 ± 0.08 b	3.76 ± 0.06 c	5.52 ± 0.04 c	7.65 ± 0.14 c
p(Pectimorf)	*0.00005*	*0.0000*	*0.00006*	*0.0000*
Interacciones				
S_1D_0	2.62 ± 0.03 ab	3.00 ± 0.01 a	4.69 ± 0.17 a	5.16 ± 0.18 a
S_1D_1	2.61 ± 0.22 ab	3.00 ± 0.00 a	4.93 ± 0.07 abc	6.00 ± 0.14 bc
S_1D_2	2.71 ± 0.08 ab	3.40 ± 0.14 ab	5.17 ± 0.09 abc	6.17 ± 0.01 c
S_1D_3	3.18 ± 0.05 bc	3.70 ± 0.11 bc	5.48 ± 0.02 bc	7.48 ± 0.02 d
S_2D_0	2.50 ± 0.29 ab	3.24 ± 0.12 a	4.65 ± 0.23 a	5.37 ± 0.15 ab
S_2D_1	2.26 ± 0.07 a	3.08 ± 0.08 a	4.92 ± 0.08 ab	6.32 ± 0.09 c
S_2D_2	2.68 ± 0.09 ab	3.41 ± 0.05 ab	5.10 ± 0.16 abc	6.59 ± 0.05 c
S_2D_3	3.48 ± 0.09 c	3.83 ± 0.04 c	5.56 ± 0.07 c	7.83 ± 0.25 d
p (substrates x Pectimorf)	*0.1940*	*0.5820*	*0.9437*	*0.9088*
Mean	2.75 ± 0.09	3.33 ± 0.07	5.06 ± 0.08	6.36 ± 0.19
Coefficient of variation (%)	9.21	4.70	4.42	3.84

* T: Orchard soil; A: Sand; CA: Rice Husk; PD: Pectimorf concentration. The level of significance (p) is shown in italics for each treatment: substrates, Pectimorf concentration, and interaction (substrate x Pectimorf). The level of significance (p) is shown in italics for each treatment: substrates, Pectimorf concentration, and interaction (substrate x Pectimorf). Means with the same letter in each data group do not differ statistically according to Tukey's test ($p>0.05$).

Shoot (S) dry weight, root (R) dry weight, S/R ratio, number of roots, and root length

There were no discernible variations in the shoot and root biomass between the substrates or with the substrate x Pectimorf interaction. Contrarily, treatment D_3, regardless of the substrate, caused an increase of (80.4; 72.7; 46.9, and 26.9%) in shoot dry weight, root dry weight, number of roots, and length, respectively (Figure 1). A significant effect of the substrate on the S/R ratio was observed, the highest average values were found in S_1 (0.428) compared to S_2 (0.330).

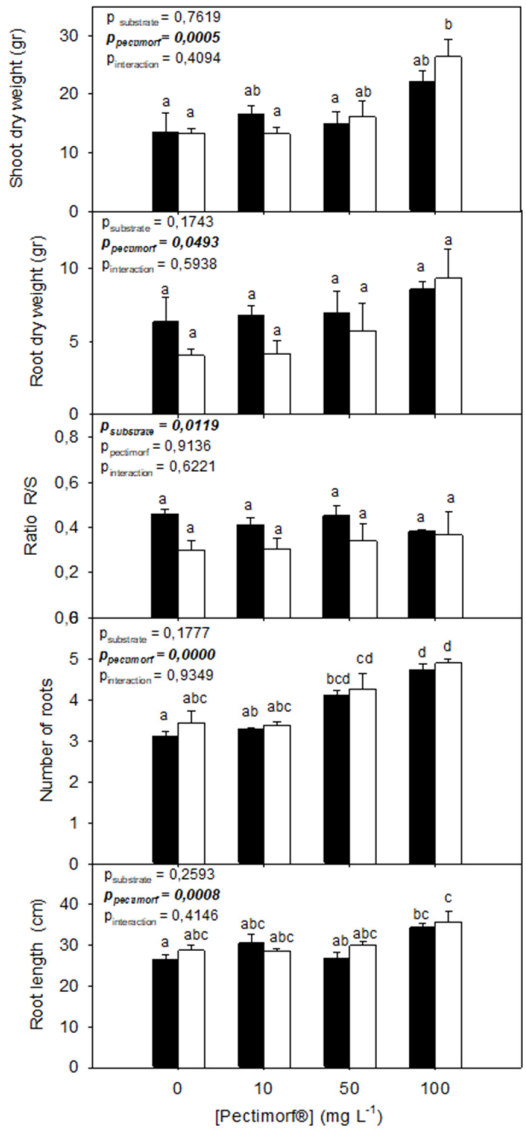

Figure 1. Shoot dry weight, root dry weight, shoot/root ratio, number of roots, and root length of cocoa seedlings subjected to 120 days of different Pectimorf concentrations in the vegetative propagation of cocoa cuttings. The EETP-800 clone was grown on two different types of substrates: 80% orchard soil 'T' + 20%

sand 'A' (S_1, black bars) and 70%T + 20% A + 10% rice husk 'CA' (S_2, white bars). Each bar shows the average of ten different seedlings ± SE (n=10). Significant differences from the two-way ANOVA (substrates × Pectimorf) are indicated by different letters above the bars. *P*-values or the significance of the effect of each factor on the response variables are indicated within each panel. The level of significance (*p*) is shown in italics for each treatment: substrates, Pectimorf concentration, and interaction (substrate x Pectimorf).

Gas exchange

No significant differences in *A* between substrates, Pectimorf treatment, and interaction (substrate x Pectimorf) were observed, with *A* values between 2.05-3.16 µmol m^{-2} s^{-1} observed. Except in the g_s which showed significant differences between the substrates (p=0.04). The mean values of E and g_s were low, 0.42 ± 0.03 mmol m^{-2} s^{-1} and 17.6 ± 1.2 mmol m^{-2} s^{-1}, respectively (Figure 2). C_i showed low values between 100 and 193 µmol mol^{-1}, showing no differences between treatments. The WUE showed values between 5.2 and 11.2 mmol mol^{-1} (very high), without significant differences between treatments (p=0.089) and between Pectimorf (p=0.279; Figure 2).

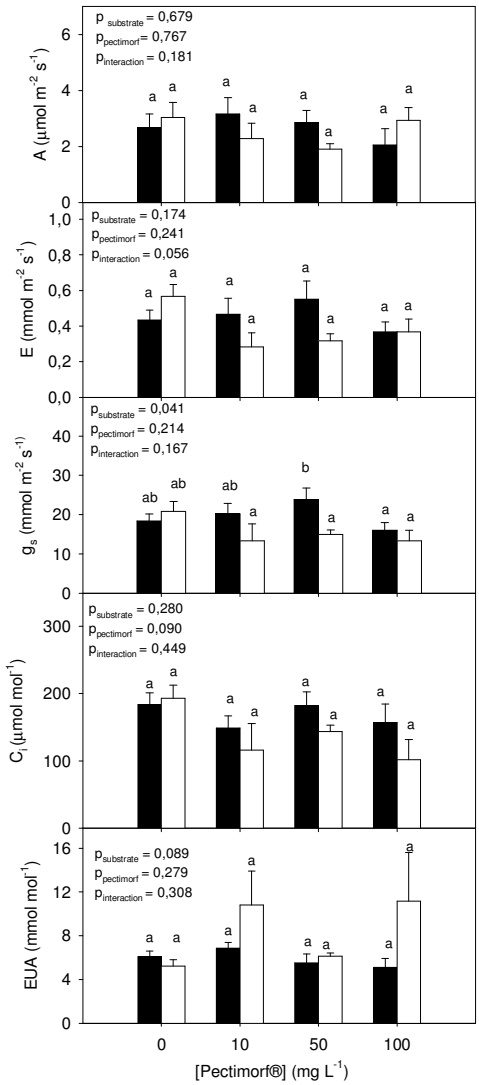

Figure 2. Gas exchange parameters: A. Net photosynthesis rate, E. Transpiration rate, g_s. Stomatal conductance, C_i. Intercellular CO_2 concentration, WUE. Water use efficiency of cocoa clone subjected to different concentrations of Pectimorf. The EETP-800 clone was grown on two different types of substrates: 80% orchard soil 'T' + 20% sand 'A' (S_1, black bars) and 70%T + 20% A + 10% rice husk 'CA' (S_2, white bars). Each bar shows the mean of six different seedlings ± SE (n=6). Significant differences from the two-way ANOVA (×Pectimorf substrates) are indicated by different letters above the bars. The p-values of the significance of the effect of each factor on the response variables are indicated within each panel.

Survival

Pectimorf concentrations of 50 mg L^{-1} and 100 mg L^{-1} markedly increased seedling survival by 1.5 and 2.1 times, respectively. When compared to untreated cuttings under comparable substrates, S_1D_3 and S_2D_3 both had the best survival rates, at 56.6 and 76.7 percent, respectively (Figure 3). There were no significant differences in the interaction (p substrate x Pectimorf = 0.6419); observing the greatest survival, regardless of the substrate at the highest concentration of Pectimorf (100 mg L^{-1}).

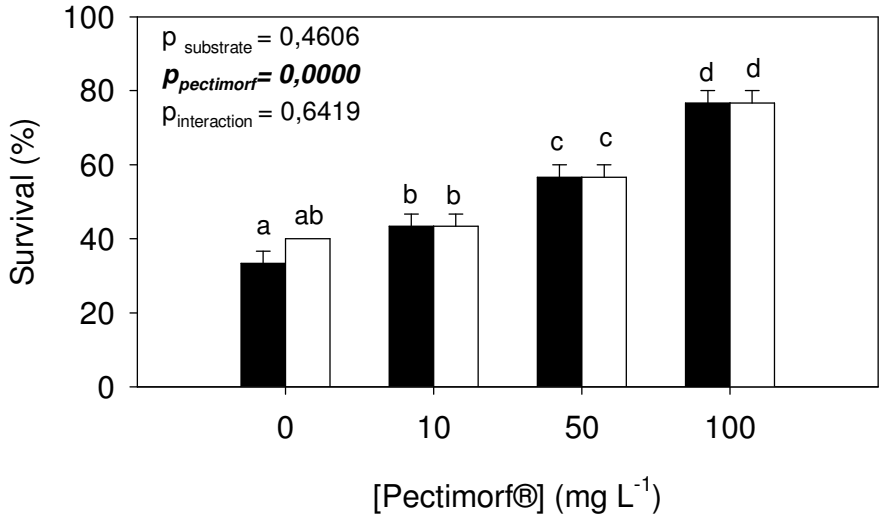

Figure 3. Survival of cocoa seedlings in response to the application of different concentrations of Pectimorf in the vegetative propagation of cocoa cuttings established in two substrates. The EETP-800 clone was grown on two different types of substrates: 80% orchard soil 'T' + 20% sand 'A' (S_1, black bars) and 70%T + 20% A + 10% rice husk 'CA' (S_2, white bars). Each bar shows the average of ten different seedlings ± SE (n=10). Significant differences from the two-way ANOVA (Pectimorf × substrates) are indicated by different letters above the bars. The p-values of the significance of the effect of each factor on the response variables are indicated within each panel.

Discussion

The results of the present research provide relevant information on two important factors for the production of cocoa seedlings, such as Pectimorf concentration, and the substrates in order to help producers, and field technicians, in the cocoa production process. The potential effect of Pectimorf as a rooting agent was demonstrated in clone EETP-800 regardless of the type of substrate, which showed a high ability to induce the formation of vigorous seedlings (greater height, diameter, dry biomass, number of leaves, and root length) with a higher survival percentage (57-77%) in the two substrates (S_1 and S_2).

Pectimorf is a hormonal chemical signal that regulates plant growth and development (Rodríguez-Izquierdo *et al*., 2021). Rizo-Alvarez *et al*. (2018) recorded an increase in growth variables in *Leucaena leucocephala* plants with the application of Pectimorf.

The potential of Pectimorf as a rooting agent has been demonstrated by the formation of adventitious roots in petioles of African violet (*Saintpaulia ionantha*) and carnation cuttings, which increases root formation and, hence better development of plants (Ramos- Hernandez *et al*., 2013). Biostimulants improve root formation and elongation, nutrient uptake, seed germination rates, and crop establishment (Shahrajabian *et al*., 2021). PectiMorf in *Manihot esculenta* positively influenced the number of roots and vigor of *in vitro*-plants (90% of the total were vigorous) (Suárez-Guerra and Hernández-Espinosa, 2015), in different plant tissues (banana (*Musa paradisiaca* L.), rice (*Oryza sativa* L.), and ornamental plants) cultivated *in vitro*, stimulated the establishment of the explants and increased the fresh mass of the calluses and favoring their transformation into embryogenic structures (Lara *et al*., 2018). The control (0 mg L^{-1}), showed a difference in all growth and developmental variables, compared to the Pectimorf application. The best results were obtained when using 100 mg L^{-1} of Pectimorf with the S_2 substrate (with rice husk) accelerating the rooting process, improving biomass and root length, and obtaining better EETP-800 cocoa plants. The results obtained in this experiment are consistent with what was reported in the banana rooting phase, where it was observed that the use of Pectimorf as a substitute for IAA in the Murashige and Skoog (MS) culture medium at a rate of 5 mg L^{-1} contributed to 100% survival and rooting of plants (Izquierdo *et al*., 2009; Izquierdo, 2013; Borges-García *et al*., 2015). Pectimorf favors growth and root formation, pericycle cell differentiation, and induction of somatic embryo differentiation processes (Lara *et al*., 2018). It must be pointed out that Pectimorf consists of oligogalacturonides, which induce greater efficiency in cell division and elongation (Iwasaki and Matsubara, 2000; Suzuki *et al*., 2002). The promotion of cell elongation appears to occur through the auxin-stimulated activation of cell wall enzymes capable of xyloglucan degradation, resulting in increased cell wall elasticity in response to increased auxin-induced turgor (McDougall and Fry 1990; Kaku *et al*. 2004).

Low values of photosynthesis, transpiration, and stomatal conductance were observed, indicating a low rate of CO_2 assimilation per water lost through transpiration, leading to high values for water use efficiency. Similarly, in other cocoa cultivars in adult trees and seedlings have been reported low photosynthesis rates (Avila-Lovera *et al.,* 2016; De Almeida *et al.,* 2018). Besides let us remember that photosynthesis measurements are instantaneous measurements, and that were only performed at 120 days from treatment, i.e. they are measurements that correspond to evaluations at an instant of time that do not necessarily correlate with what is observed in the growth variables that are an integral measure of biomass accumulation over time due to photosynthesis. It has been widely reported that cocoa is a shady species that grows in understory environments of tropical forests with high rainfall and reduced light availability. Cocoa shows a low light saturation point, low maximum photosynthesis rates at saturating light, low respiration rates in darkness, and high quantum efficiencies (Ávila-Lovera *et al*., 2016; De Almeida *et al*., 2018). The values of A, g_s, and E observed in this study are lower than those reported in National cocoa clones in San Agustín, in Pichilingue, and PMA 12 and CCN 51 evaluated in Quinindé and San Agustín, respectively (Tezara *et al*., 2020; Jaimez *et al*., 2022).

There were no significant differences between the two substrates in the variables studied. However, the seedlings grown in the S_2 substrate were larger, with a greater number of leaves, a higher S/R ratio, and with lower stomatal conductances (g_s), which would result in less stomatal opening and therefore less water loss by transpiration, whereby the physiological performance of plants is improved. The S_2 substrate contains rice husk, which elicited superior growth and physiological response. Rice husk is characterized by a high silica content, which allows preserving the physical and chemical properties of the soil for long periods (Vélez Carvajal *et al*., 2014).

Conclusions

Application of Pectimorf at 100 mg L^{-1}, represents the most effective treatment for increasing cocoa seedling height, stem diameter, and dry biomass, due to better root development, showing the high inducing potential of the bioregulator as a rooting agent in EETP-800 cuttings. The S_2 substrate (70% soil + 20% sand + 10% rice husk) showed results encouraging in obtaining plants with a slightly higher development at the end of the experiment. Totally, it is recommended to use the S_2 substrate with 100 mg L^{-1} Pectimorf for producing high and healthy vigor cocoa seedlings.

Authors' Contributions

Research, Conceptualization, Validation, Project Management, Fund Acquisition: JJRP. Writing: proofreading and editing: WT. Research, methodology: LTLLR. Writing: Original Draft Preparation, Writing: Revision: JATR. Data curation, data analysis: VHRCH. Writing, revision, formal analysis: LGHM. Review and Editing: SF.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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