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TECHNICAL NOTE

WATER DEFICIT INFLUENCE UPON POLLEN GRAIN PRODUCTION IN CACAO GENOTYPES (*Theobroma cacao*)

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ASTRACT

Genetic improvement programs in cacao (*Theobroma cacao* L) have focused on obtaining new varieties with high yield and resistance to diseases. However, drought tolerance response has not been considered enough in order to face the climate change emergency. Events such as prolonged droughts or excessive rainfall can affect the pollen production of cacao flowers and others reproductive characteristics. Hence, this study was proposed to learn about the hydric deficit influence on pollen production of four Ecuadorian cacao genotypes. The results indicated statistical differences between CCN-51 and the "National" clones ($P \leq 0.05$). CCN-51 reached the highest pollen production values in the wettest treatment (26,546 ±100) compared to the "National" clones, which had no differences among them. There was a positive correlation between pollen production and the highest irrigation treatments (r=0.78), observing that while the water level was the highest, the pollen grain formation (PGF) increased, and the opposite when the water level diminished. It is concluded that the adequate water amount in the soil can increase pollen production. However, it is essential to emphasize the strong genetic influence on drought tolerance traits that could be severely affected by climatic changes. The CCN-51 and EET-575 clones should be considered when planning to develop drought-tolerant hybrids of cacao.

Additional keywords: Breeding, drought conditions, pollination, reproduction

RESUMEN

Influencia del déficit hídrico sobre la producción de granos de polen en genotipos de cacao (*Theobroma cacao*)

Los programas de mejora genética en cacao (*Theobroma cacao* L) se han centrado en obtener nuevas variedades con alto rendimiento y resistencia a las enfermedades. Sin embargo, poco se ha considerado su tolerancia a la sequía para enfrentar la emergencia del cambio climático. Eventos como las sequías prolongadas o la lluvia excesiva pueden afectar la producción de polen de flores de cacao y otras características reproductivas. Por lo tanto, en este estudio se propuso conocer acerca de la influencia del déficit hídrico en la producción de polen de cuatro genotipos ecuatorianos de cacao. Se encontraron diferencias significativas entre el clon CCN-51 y los clones "Nacionales" ($P \leq 0{,}05$). CCN-51 alcanzó el valor más alto de producción de granos de polen (26.546 ± 100) en el tratamiento más húmedo, comparado con los clones "Nacionales", los cuales no mostraron diferencias entre sí. Hubo una correlación positiva (r=0,78) entre la producción de polen y los volúmenes de agua aplicados, donde los niveles altos de riego se correspondieron con la mayor formación de granos de polen (FGP) y cuando el nivel de riego fue mínimo, la FGP cayó drásticamente. Se concluye que las cantidades adecuadas de agua en el suelo pueden aumentar la producción de polen. Sin embargo, es esencial enfatizar la fuerte influencia genética en los rasgos de tolerancia a la sequía que podrían verse gravemente afectados por los cambios climáticos. Los clones CCN-51 y EET-575 deberían considerarse al planificar desarrollar híbridos de cacao tolerantes a la sequía.

Palabras clave adicionales: Mejoramiento, polinización, reproducción de cacao, sequía,

INTRODUCTION

Climate change brings severe consequences to the agricultural sector worldwide, causing millionaire losses in many agricultural regions (Macías et al., 2019). Some cacao genotypes have been shown to be sensitive to prolonged drought periods (Ávila et al., 2015).

Manabí province, in Ecuador, is a world leader in "fine cacao" production, but it has been severely affected by climate disorders, such as "El Niño" phenomenon in 2016-2017 (Macías et al., 2019; Vera et al., 2021), which caused floods and prolonged droughts in some places of Ecuador (Zakariyya et al., 2017). This causes a negative impact on cacao yields, because of the affection of the main processes involved in reproductive efficiency, such as pollen flow (Mena et al., 2020; García et al., 2020a).

The process of pollen formation requires a high water availability for pollen hydration and nutrition (Ávila et al., 2015). Also, other floral

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structures, such as filaments, are essential in transporting water and nutrients to pollen-bearing teak, which allows the passage of essential substances involved in the turgor processes of the pollen cell wall (Pacini and Dolferus, 2019). On the contrary, when there is not enough water the pollen grains dry out because of not being able to store water in their vacuoles, resulting in their dissection and death; in other cases, they lose viability, becoming unable to germinate (García et al., 2015; Jalca et al., 2019; García et al., 2020b).

Based on these considerations, this research was conducted with the purpose of determining the influence of the water availability in the soil upon the production of pollen in cacao, establishing a relationship between the water deficit and the pollen production. This could contribute to the identification of new parental clones with drought tolerance traits, a key element in the designing of new hybrids that adapt to hydric disorders and other climate challenges.

MATERIALS AND METHODS

The research was carried out at the Experimental Campus "Teodomira" of the Technical University of Manabí (UTM), located in the Santa Ana canton, Manabí province, in the Portoviejo river basin (80º26′22″ W, 01°04′15″ S, 60 m.a.s.l.). A summary of the weather data during the experimental period is presented in Table 1.

The counting of the pollen grains was performed on a group of cacao plants that had been submitted to different irrigation treatments in a previous study from July 2017 through December 2018

Table 1. Average weather data during the 2017-2018 at the Teodomira Agricultural Station, Manabi Technical University (UTM)

Year	Temperature		Relative Humidity		Wind speed	Radiation	Rainfall
	Min	Max	Min	Max			
	(ºC)		(%)		($m \cdot s^{-1}$)	($MJ \cdot m^{-2} \cdot d^{-1}$)	($mm \cdot year^{-1}$)
2017	19.6	33.7	76	84	1.7	20	985.0
2018	19.6	34.0	72	84	1.9	20	719.3

Source: Instituto Nacional de Meteorología e Hidrología (INAMHI–2018)

Plant material. The four cacao clones studied were EET-103, EET-575, and EET-576 belonging to the "National" type (Forastero genetic group; fine flavor cacao), and CCN-51 (Trinitarian genetic group) (Loor et al., 2009; Loor et al., 2012). They were grown in containers 0.82 m high and 0.27 m^3 in capacity, filled with a substrate mix composed by 60 % of a clay loam soil, 20 % coarse sand, and 20 % organic matter. At the time of evaluation, the plants averaged 1.5 m tall, with a mean root depth of 0.5 m.

Experimental design. A completely randomized block design with a split-plot arrangement with four replications was used in the experiment. The main plots consisted of the four different moisture levels, and the subplots were the four cacao clones.

Experimental procedure. Plants were kept in an open place, sheltered with a provisory roof consisting of a plastic coverage (83±5 % light transmission) placed at 6 m high, to avoid the influence of rain, and ensuring the desired water sheet.

The cacao plants had been receiving irrigation as follows: the amount of irrigation water applied to the wettest treatment was about 3 $L \cdot day^{-1}$ (determined as 100 % of the water requirement), so the accumulated volume in the period of 18 months was 1609 L. The other treatments consisted in fractions of 0.8, 0.6 and 0.4 of that volume (Table 2).

Table 2. Water volumes applied to cacao plants according to the established irrigation fractions

Treatment identification	Fraction of irrigation	Cumulative water volumen (L)*
WS_{100}	1.0	1609
WS_{80}	0.8	1287
WS_{60}	0.6	965
WS_{40}	0.4	644

*Cumulative from July 2017 to December 2018

Counting the number of pollen grain per flower (PGF). It was measured by direct count, with the following procedure: forty pre-anthesis flowers (buds) per clone were collected at the middle stratum of cacao plants (approximately at 70 cm from the soil) in December 2018. Flower buds were conserved in FAA solution prepared with formaldehyde, glacial acetic acid and 70 % ethanol (5: 5: 90 v/v/v), until its usage. The five anthers of the cacao of each flower were carefully removed and placed in a solution of 100 µL distilled water: methylene blue: glycerin (7:1:2) in glass tubes. Anthers were then smashed using a glass stick, and 1 µL was transferred into a Neubauer chamber (Marienfeld mod. 0610130) and an optical microscope (Motic mod. BA4103) was used for counting. Each reading was multiplied by 100 to obtain the number of pollen grains per flower.

Statistical analysis. The analysis of the PGF and the interactions between clones and water volumes were evaluated by variance analysis (ANOVA), and significant effects of the treatments compared by the Tukey test. The degree of association between the PGF and the four water treatments was obtained by correlation analysis. In all cases, SAS software 9.4 (SAS Institute, Cary, NC, USA) was used.

RESULTS AND DISCUSSION

Cacao genotypes exhibited different responses when receiving different water volumes. In the wettest treatment (WS_{100}), there were significant differences between the National clones and CCN-51 ($P \leq 0.05$) (Figure 1A). CCN-51 showed the highest PGF of 41225 ± 831. This clone was the earliest and most productive material in tropical dry forest conditions (Escobar et al., 2020). The rest of clones had similar performance among them, with EET-103 showing a low mean with a count of 21285 ± 1518.

In the second water treatment (WS_{80}), the general PGF values were decreasing, and the clone CCN-51 exhibited a large drop (Figure 1B), thus there were no significant differences among the four clones (P>0,05). The PGF values ranged between 25864 ± 2444 in CCN-51, and 21596 ± 1299 in EET-576 clone.

In the WS_{60} treatment, the PGF values continued decreasing, and the clone EET-576 presented the lowest number of pollen grains with a value of 9232 ± 754, significantly lower ($P \leq 0.05$) than the other three clones (Figure 1C). These ranged between 24947 ± 1825 in CCN-51 and 19003 ± 1945 in EET-575, without differences among them.

The driest treatment (WS_{40}) promoted the lowest scores of PGF for the four clones. And again, the clone EET-576 was significantly lower than the other clones (Figure 1D).

Differences observed regarding pollen production in the four commercial cacao clones can be attributed to their genetic background. On the other hand, when considering the interaction between clones and irrigation volumes an important interaction ($P \leq 0.001$) was observed. For example, CCN-51 presented a large decrease in pollen production when going from WS_{100} to WS_{80}, while EET-575 displayed a more stable decrease, reflected by a steady slope (Figure 2). This indicate that different cacao genotypes behave as less sensitive to changes in soil moisture (i.e. EET-575), whereas CCN-51 being the clone with the highest pollen production was greatly affected when soil water dropped from full irrigation. Additionally, the clone EET-103 showed, in general, a low pollen production, but this was maintained at different soil water contents, and was only affected when the water availability decreased to very low values (40 %).

It was, also, observed that when the water volume diminished from WS_{80} to WS_{60} %, the PGF's of CCN-51 and ETT 103 remained relatively constant, while the clone EET-576 was notoriously affected and tended to decrease its PGF. When the water volume dropped to WS_{40}, pollen production decreased in all clones (Figure 2).

This behavior could be attributed to the energy demands and water requirements that the anthers have to develop viable pollen grains (Ávila et al., 2015). Low soil moisture levels could affect the degree of pollen dehydration in cacao, and its rehydration in the stigma (Vera et al., 2021). Pollen grains store water in vacuoles where its content is generally greater than 60 % during development (Pacini et al., 2011); in the formation of recalcitrant pollen types in species of Malvaceae, such as cacao, they present sensible desiccation at less than 30 % water content (Pacini and Dolferus, 2019).

It has been mentioned that pollen production depends on the genetic group and on the genotype *per se* (García et al., 2017; Gupta et al., 2018); besides, it could be indirectly influenced by the weather conditions. In fact, other authors support as well that pollen production is related to some abiotic factors related to water availability, such as prolonged drought periods, and irregular distribution of the rainfall throughout the year (Lorna et al., 2019; Zhang and Steiner, 2022).

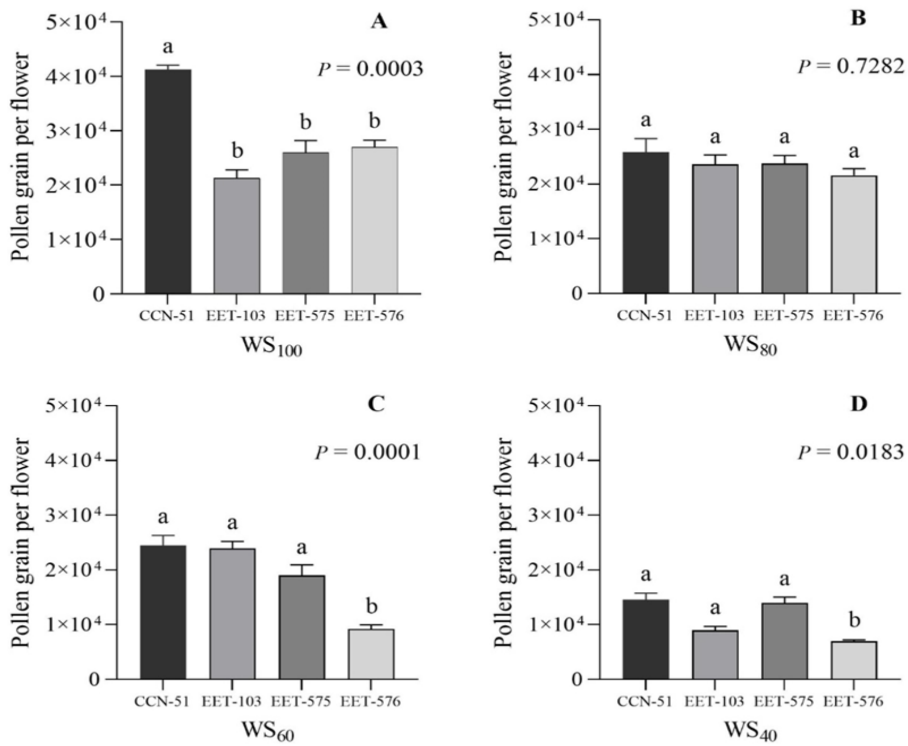

Figure 1. Production of pollen grains per flower of *Theobroma cacao* L. National type and CCN-51 clones under four irrigation treatments (function of full irrigation): A: 100 %. B: 80 %. C: 60 %. D: 40 %. Columns with distinct letters differ statistically according to the Tukey Test ($P \leq 0.05$). Vertical lines represent standard error

Moreover, it has been shown that when the rain periods are irregular, it can negatively alter the pollen flow, due to both excess or water deficit in "National" and "Trinitarian" cacao types (Mena et al., 2020). Our study did not evaluate the rain effect on pollen production, but we could associate the volume of annual rain and the volume of water added to the treatments. This can be supported by a study conducted on the Ecuadorian coast, where the pollen production was higher from December to April, corresponding to the rainy season, showing a high correlation between both factors (García et al., 2020).

In related cases, when crop transpiration has been very high, the viability and pollen production can be drastically reduced (Pacini and Dolferus, 2019). This behavior is related to our results, where a significant correlation (r = 0.78; $P \leq 0.05$) between PGF and the amount of applied water was found, observing that at the greatest total water volume (1609 mm), the number of PGF was high (PGF = 28,863). On the contrary, when total volume was low (644 mm), the number fell to 11,126 (Figure 3). As shown, PGF decreased consistently as the irrigation volumes were lower.

In this sense, Escobar et al. (2022) mention that water deficit can affect flowering and the compatibility between clones by reducing flower formation and pollen viability, reducing fruit set and formation. All processes involved in pollen formation are linked to individual genetic traits (García et al., 2019). This is supported by Morgan (2000), who mentions that the osmotic adjustment of pollen grains has been used to identify genetic variation for drought tolerance.

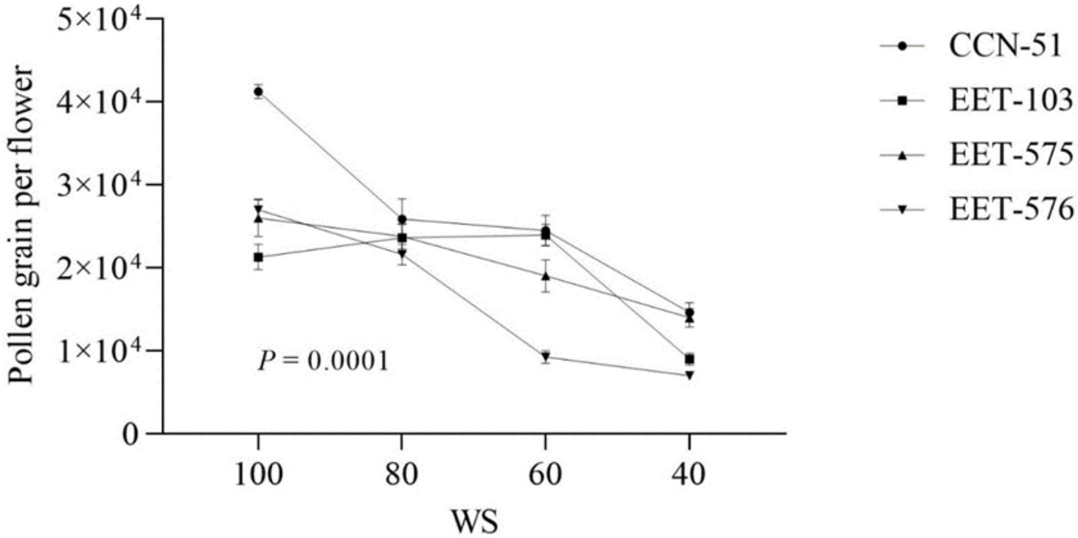

Figure 2. Interaction between production of pollen grains per flower (PGF) of *Theobroma cacao* L. clones and the percentage of the water volume (WS) applied. Vertical bars represent standard error

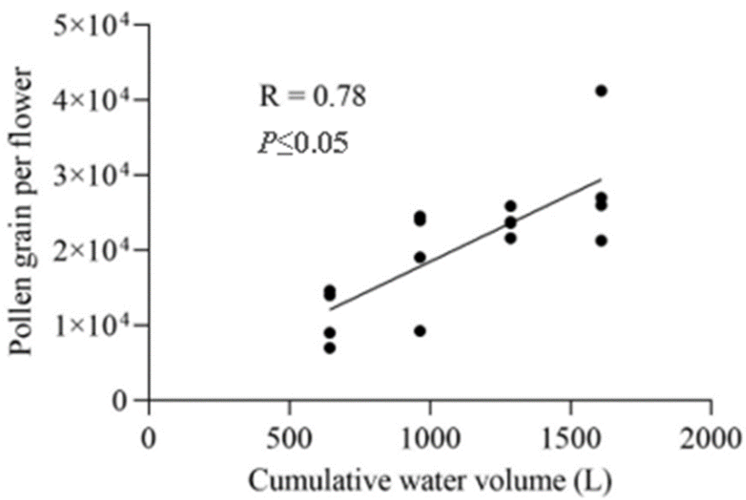

Figure 3. Correlation between production of pollen grains per flower (PGF) of *Theobroma cacao* L. clones and the cumulative water volume in a period of 18 months.

CONCLUSION

Pollen grain production is strongly influenced by the availability of water in the soil, even though the genetic component is very important. CCN-51 clone was outstanding as a pollen grain producer among the studied genotypes, and EET-575 clone showed to be, only partially affected by the water deficit. Therefore, it is suggested that both clones could be used as progenitors when searching for tolerant to long drought period hybrids because these clones developed a substantial number of pollen grains per flower even under restrictive soil moisture; such clones could be recommended in areas with low annual rainfall and those affected by droughts due to the disturbances caused by climate change.

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