Hormonal profile in *Theobroma cacao L.* plants grown in soils with high cadmium level and treated with heavy metal immobilizing amendment.

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Abstract

Lo hacemos al final

Key words: cocoa plants, phytohormones, cadmium plant responses.

Introduction

The production of cocoa, *Theobroma cacao* L., is one of the most important crop in Peru because it generates high incomes for the country and the farmers who dedicate themselves to this crop are medium and small producers in rural areas (MINAGRI, 2018). Due to this, it is essential to carry out research that solves the lack of quality plant material for planting and that guarantees sustainable production over time. In this sense, private and state institutes and companies are making efforts to improve cocoa production.

There are many climatic and soil conditions and demands that influence the development of *T. cacao* cultivation. It is a plant that requires field capacity in the soil, the optimum rainfall it needs to develop and produce is 1500 to 2000 mm of annual rainfall, the optimum minimum temperatures 21°C and maximum 32°C, given these conditions the plant It will have good growth, with abundant flowering and fruiting. Relative humidity should be between an average of 70% and 80%, this condition causes sanitary problems, very strong winds can break branches, so they should be light and not come too close to the sea because they can be loaded with chlorides. sodium and other salts that can be harmful to the crop. The altitude that optimally develops cocoa cultivation is from 30 meters above sea level to 800 meters above sea level. The soils for their normal development must have a depth of 1.50 meters and contain main elements that must be in adequate amounts in usable form such as calcium, nitrogen, phosphorus and potassium with good drainage and high in organic matter with a pH between 6 .5 - 6.8 and preferably not very clayey (Pinto, 2012). COLOCAR CARACTERISTICAS DEL SUELO CON CADMIO EN UNA SOLA DE LAS REGIONES la que no está cerca de la selva) Y MENCIONAR LA ENMIENDA UTILIZADA COMO POSIBILIDAD PARA QUE LAS PLANTAS PUEDAN CRECER EN ESA ZONA

Under these environmental and soil conditions, plants produce substances or regulators that are synthesized and that intervene in growth and development plant processes. These substances are known as phytohormones, which include abscisic acid (ABA), auxins (AIA), salicylic acid (SA), jasmonic acid (JA), gibberellins (GA3) and zeatin (ZEA). The identification of these substances and quantification in different plant tissues could provide very useful information to understand the responses at the level of plant promotion and development, under favorable or unfavorable environmental conditions. Therefore, the aim of this study was to analyze the hormonal profile of *Theobroma cacao* plants grown in soils with high cadmium levels and treated with a heavy metal immobilizing amendment.

1. Materials and Methods

1.1 Plant material and growth plant conditions

Theobroma cacao L. seedlings were used in this study (colocar variedad de cacao q se utilizó). Seedlings were grown in a chamber with a cycle of 16 h light (200 µmol m-2 s-1) at 28°C and 8 h darknes at 20°C, and with 70% relative humidity (colocar condiciones de la camara de cultivo o invernáculo donde estuvieron las plantas hasta el traspaso). Thirty six days of culture *Theobroma cacao* L. seedlings were placed in cultivation area XXX (especificar area de cultivo, caracteristicas del suelo) and each group was divided into two subgroups, treatment and control.

1.2 Treatments

The aforementioned heavy metal immobilizing amendment was applied to the treatment group (poner caracteristicas de la enmienda segun product PSW, lo que se pueda mencionar a grandes rasgos) Samples were collected 4 months after transplantation in the cultivation area and they were transported at -70°C to the laboratories where the samples were stored in liquid nitrogen, then dehydrated by lyophilization and stored until analysis of hormones.

2. Determination of growth parameters

Root length and shoot height (poner parametros de crecimiento que se tengan , biomasa?) were monitored throughout the experiment. Measurements began at the same time as the application of the treatments (when the plants had undergone XXX days of culture). Growth parameters were measured in XXX control and treated plants.

3. Hormone profile analysis

Dry weight (100 mg) corresponding to leaves, stems and roots were collected and homogenized with liquid nitrogen. 1 ml of extraction buffer (distilled water at pH 2.8-3) was added and deuterated ABA, SA, GA3, ZEA, JA and AIA were used as internal standard (J.D. Chen, USDA- ARS of Beltsville, MD, USA). The sample was then centrifuged at 12.000 rpm

for 8 min, and the supernatant was removed and placed in a new tube. 1 mL of ethyl acetate was added and the organic phase was taken up again. Subsequently, it was evaporated to total dryness and the extracts were taken up with 100 µl of MeOH (100%), placed in specific vials containing 150 µl capacity inserts, injecting an aliquot of 10 µl of each sample into the equipment. Chromatographic runs were performed on an Alliance (Waters 2695) liquid chromatograph (LC) with autoinjection and UV detector, coupled to a Micromass Quattro Ulttima Pt tandem mass spectrometer (MS-MS), monitored using MassLynx V4.1 software. Finally, the data was expressed as ng of compound/g DW of sample.

Results

Hacer graficos de crecimiento y hormonas en una sola zona (uno por cada hormona)
GRÁFICO ILUSTRATIVO DE LAS PARTES DE ÓRGANOS QUE MOSTRÓ ANGEL

Discussion

It has been shown that abiotic stress activates different response mechanisms in the plant to ensure its persistence, including hormonal regulation as in the case of salicylic acid (Benková, 2016; Khan et al., 2013). In the case of cadmium, it is stored in the leaves, where it affects the photosynthetic process by altering the electron transport chain (Liu et al., 2011). Several studies have observed the importance of salicylic acid in the response to biotic and abiotic stress (White, 1979; Mohamed, et al., 2020). Likewise, in the case of abscicic acid, it has been shown that it acts as a signal mediator in the adaptive response of plants to different stress conditions (Sah, et al., 2016). In the present study it can be seen that the levels of salicylic acid and abscisic acid are lower when applying the amendment than when not applying it. In this case, the studies were carried out in the cultivation areas where all the experimental units were subjected to a high level of cadmium in the soil, above 1ppm. The decrease in the synthesized levels of the mentioned hormones could be due to the fact that the

application of the amendment decreases the cadmium that is bioavailable in the soil, preventing its passage to the plant (Zheng et al., 2020). One of the components present in the amendment is silicon, which has been observed that the presence of this element in the soil can form complexes with different heavy metals, thus generating its inerting (Piri et al., 2020). On the other hand, the organic matter provided by the amendment may be generating a similar effect by complexing this metal and preventing the plant from assimilating this heavy metal, reducing the synthesis of hormones in response to stress in the plant (Pinto et al., 2004). Another mechanism of action that could explain the results obtained is the contribution of calcium by the amendment. In several studies it has been observed that cadmium is a metal that competes with calcium transporters in the plant. By increasing the levels of available calcium, the assimilation of cadmium by competition is reduced, generating a better physiological state in the plant (Eller et al., 2015). From the results obtained, it can be concluded that the application of the amendment has an effect on the levels of jasmonic acid and abscisic acid, which are phytohormones related to the response to stress. This observed effect could be due to a possible ability of the amendment to reduce bioavailable cadmium in the soil, improving the agronomic conditions for the development of cocoa (Theobroma cacao) cultivation