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Influence of low light intensity and soil flooding on cacao physiology

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ABSTRACT

Growth and development of plants frequently are limited by multiple abiotic stresses that occur simultaneously in the environment. 'Cabruca' an agroforestry system is a main cropping system invariably adapted for cultivation of cacao in southern Bahia, Brazil. In this system of management, cacao is grown under the shade of native species of the Atlantic Forest. However, growing cacao under the shade of trees is still questionable due to higher incidence of pests and diseases and difficulty of evaporation of excess soil water in high rainfall periods that often causes flooding. The objective of the current study is to evaluate the performance of two clonal genotypes of cacao, contrasting for tolerance to soil flooding (TSA 792 – tolerant and TSH 774 – intolerant), subjected to four levels of light intensity and two water regimes, control and flooded, in order to elucidate the mechanisms of tolerance to these stresses under 'Cabruca' environment. All the plants survived the period of flooding, however, with flooding stress visual symptoms like leaf chlorosis, lenticels hypertrophied on the stem base, and decomposition of roots were observed. Obtained results demonstrated morpho-physiological and molecular changes in response to low light intensity and low light intensity × soil flooding interaction. Significant effects ($p < 0.05$) of isolated factors, double interaction and inter- and intra-genotypic differences ($p < 0.05$) were observed for leaf gas exchange, chlorophyll fluorescence, and growth parameters. Differences among light intensities, water regime, plant organ ($p < 0.001$), and for the interactions low light intensity × water regime and water regime × plant organ ($p < 0.01$) were observed with respect to total soluble sugar content. Isolated factors and double and triple interactions ($p < 0.01$) had significant effects on the starch accumulation. Triple interaction was also observed in the expression of genes coding for ADH, LDH and PDC enzymes and for aquaporin PIP1;2. Low light intensity interacts with soil flooding, limiting the damages caused by flooding in cacao plants under lowest light intensity. Flooding was the dominant factor for the most physiological responses observed during the interaction between light intensity and soil flooding whose physiological adjustments were different amoung the clonal cocoa genotypes assessed.

1. Introduction

Theobroma cacao L. (cacao tree) is an economically important crop in many tropical countries being grown on about 9.7 million ha of land area with an estimated production of 4.5 million tons cocoa beans/year (FAOSTAT, 2016; ICCO, 2016). Brazil is the world's 6th largest producer of cocoa preceded by Côte d'Ivoire, Ghana, Indonesia, Nigeria and Cameroon. In cacao production systems, shading stands out as one of the most important cultural techniques in maintaining the ecological conditions needed for growth, development and production of plants (Cunningham and Burridge, 1960). Thus, cacao trees are usually intercropped with other native and or introduced tree species whose main purpose is to provide shade from the establishment to the production phase.

In southern Bahia State, Brazil, 70% of the produced cocoa is grown in agroforestry system called 'Cabruca' in which the understorey is suppressed and the cacao is established under the shade of thinned Atlantic Forest (Lobão et al., 2007).Technical recommendations for the cocoa crop in the 1960-70 decade were thinning of native trees to 25–30 trees/ha (Alvim, 1966) where shading should permit passage of 50–60% of light intensities for mature cacao trees (Alvim, 1977; Gramacho et al., 1992). However, Araujo et al. (2013)

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observed a high heterogeneity in the density of native trees in cropping systems classified as cacao-'Cabruca' systems. Further they reported that in southern Bahia according to the number of residual trees/ha these systems were characterized as follows: 'Cabruca' *stricto sensu* with ≥ 50 trees/ha; 'Cabruca' *intermediate* with 21–49 trees/ha; and 'Cabruca' *sparse* with ≤ 20 trees/ha. Hence, cacao trees in Cabruca systems are grown under different light intensities depending on the tree density. The major benefits of agroforestry systems consist of soil conservation, better utilization of water resources and erosion control; longevity of the plantations; higher production stability; reduction of invasive plants; diversification of the farm with use of trees of economic interest; better nutrient cycling and reduction of nutrient imbalances (Beer, 1987; Beer et al., 1998; Hartemink, 2005; Young, 1997). In cabruca systems wind speed and evapotranspiration are reduced, and minimizes soil extreme temperature variations which are important for young cacao plants that are sensitive to desiccation (Leite et al., 1980).

Cacao is considered tolerant to shade since it develops better under moderate shade and has physiological characteristics that are those of a shade-adapted species as low irradiance saturation (Mielke et al., 2005), low photosynthetic rate and high sensitivity to photoinhibitory stress (Acheampong et al., 2013). According to Raja Harun and Hardwick (1987), when cacao leaves were exposed to 50% of the irradiance saturation, photosynthesis rate declined only after 4 h of exposure and markedly decrease with the gradual increase in irradiance. But, when leaves were exposed to a light intensity 100% higher than the irradiance saturation was observed a decrease in photosynthetic rate immediately after the start of exposure. The authors demonstrated that cocoa leaves undergo photoinhibition even when grown under light intensities below the maximum solar irradiance, affecting growth and productivity. In some species, very high light intensities cause decline in photosynthesis (Kozlowski, 1957) mainly in leaves of species adapted to shade. Nevertheless, the use of trees for shaded cacao has become controversial given the fact that in these conditions, in addition to competition for water (Bonaparte, 1975), there is a higher incidence of pests and diseases and a cocoa yield decrease when compared to growing under full sun (Alvim, 1958; Ahenkorah et al., 1974; Ahenkorah et al., 1987; Almeida and Valle, 2007). Recently Andres et al. (2016) state that in addition, agroforestry systems provides multiple benefits and contribute to a wide array of ecosystem services such as: improved pollination, long-term stable cocoa yields, longer lifespan of cocoa plantations, control of pests and diseases, erosion control,biodiversity conservation and enhancement, climate change mitigation through C sequestration, nutrient cycling, soil fertility maintenance or enhancement, watershed protection and reduction of deforestation

In the southern Bahia, location of the main remnant of the Atlantic Forest in which 'Cabruca' is the dominant matrix, rainfall shows a gradient that decrease from the coast towards the interior, and from north to south, with annual totals exceeding 1200 mm and reaching over 2000 mm near the coast (Sambuichi et al., 2012). Cacao cultivation requires deep soils with good water holding capacity, containing adequate levels of water and nutrients and high organic matter content. Southern Bahia soils are fertile, moderately deep and are classified as Alfisols, Ultisols, Oxisols, Alluvial and Hydromorphic. In cacao-'Cabruca' systems, the higher availability of organic matter is beneficial because promotes infiltration of water and nutrients into these soils. However, in periods of high rainfall the soils are easily saturated with water, and waterlogging and temporary flooding are often observed in this environment (Almeida and Valle, 2007).

During the flooding process, excess water replaces the air present in the soil pores, restricting oxygen flow into the soil and creating a condition of hypoxia (oxygen deficiency) or anoxia (total absence of oxygen) (Sauter, 2013). Plants intolerant to this condition show a reduction in photosynthetic activity due to low internal concentration of CO_2 in the leaves caused by stomatal limitation. For relatively long periods of flooding, restrictions in the photosynthesis that do not occur by stomatal limitation can be attributed to damage in enzymes of the Calvin cycle, degradation of photosynthetic pigments, reduction of leaf water potential, as well as deterioration in distribution of photoassimilates due to low activity of absorption (Kreuzwieser and Rennenberg, 2014).

Changes caused by hypoxia/anoxia promote biochemical, metabolic and morphological changes and modify the translocation of metabolites from the root to the shoot (Kreuzwieser and Rennenberg, 2014). Moreover, responses like changes in ATP production and protein synthesis, and an increase in mRNA transcript associated with anaerobic response are observed (Voesenek and Bailey-Serres, 2015). Under hypoxia, photoassimilates transport in the phloem is inhibited and the translocation of carbohydrates to the root system decreases. Furthermore, oxidative phosphorylation and glycolysis is inhibited and fermentation pathway is activated promoting the accumulation of products as ethanol, lactate, alanine, Gaba, succinate and malate (Fan et al., 1997) where the ethanolic fermentation is more efficient than lactic acid fermentation in maintaining the survival of the species to the stressful conditions. In hypoxic/anoxic conditions, there is induction of expression of genes encoding anaerobic proteins (ANPs) and immediate repression if there is reoxygenation of the medium (Bailey-Serres and Colmer, 2014).

Flooding is a major barrier to early growth and establishment of the cocoa in places subject to periodic flooding as in case of Brazil, Ghana, Nigeria and Ivory Coast where occasionally the total precipitation exceeds evapotranspiration and soil becomes hypoxic (Almeida and Valle, 2007, 2009; Sena Gomes and Koslowsky, 1986). To better understand the mechanisms of tolerance to flooding by cacao, Bertolde et al. (2010) submitted 35 cacao genotypes to 45 days of soil flooding and distinguished TSA 292 as flood-tolerant and TSH 774 as flood-intolerant genotype. Afterwards, Bertolde et al. (2012) and Bertolde et al. (2014) showed the physiological and biochemical characteristics, the gene expression and activity of enzymes involved in the anaerobic fermentation pathway, and identified proteins differentially expressed in the metabolism of TSA 792 and TSH 774 genotypes during the stress by O_2 deficiency in the soil. However, these authors only reported the flooding effects, without regards that the cacao grown in most farms occurs in shaded environment with incidence of low irradiance. In view of this fact, the aim of this study was to test the effect of the flooding associated with low light intensity on the performance of cacao genotypes tolerant and intolerant to flooding, under the hypothesis that the low light intensity, similarly to the light intensity observed in 'Cabruca' environment, can mitigate the damage caused by soil flooding.

2. Materials and methods

2.1. Plant material and growth conditions

Cacao clonal genotypes contrasting for flooding tolerance [TSA 792- tolerant and TSH 774 – intolerant (Bertolde et al., 2010)] were evaluated. These two genotypes were provided by the Instituto Biofábrica do Cacau (IBC – Banco do Pedro, Ilhéus, BA, Brazil) with approximately 4–6 months after the rooting of stem cuttings. The stem cuttings were obtained from the plagiotropic branches of matrix of five to ten-year-old plants. Cuttings were allowed root in 288 cm^3 black polyethylene tubes containing organic substrate (Pinus bark + coconut fiber, at a 1:1 ratio) enriched with macro and micronutrients minerals, according to the crop requirements (Souza, 2007; Souza and Carmello, 2008, 2011).