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Genetic variation in bioaccumulation and partitioning of cadmium in *Theobroma cacao* L.

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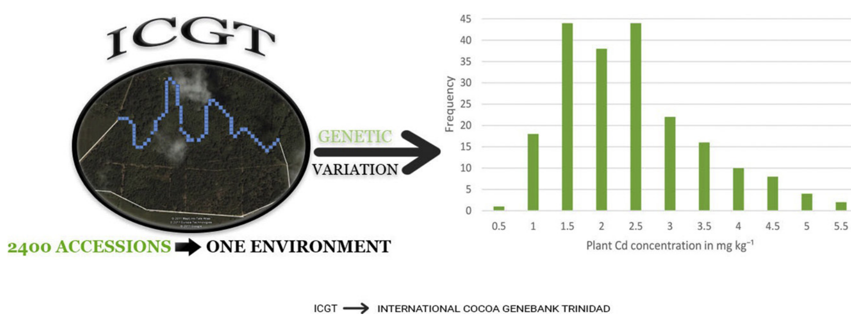
HIGHLIGHTS

- Cadmium accumulation in cocoa beans varied 13-fold across 77 cocoa genotypes.
- Differential partitioning of cadmium between the reproductive and vegetative tissues.
- 18%–56% of the total Cd mass in the bean was found in the testa.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) is a non-essential heavy metal that is toxic to both plants and animals and chocolates have been identified as a contributor to the human dietary Cd intake. One hundred accessions representing the various genetic groups and hybrid populations in *Theobroma cacao* L. held at the International Cocoa Genebank, Trinidad were evaluated for leaf and bean cadmium levels with three tree replications. Representative samples of soil from the drip zone around each tree were evaluated for bioavailable cadmium. Although there were significant differences ($P \le 0.05$) among genetic groups for leaf and bean Cd much of the variation was between accessions. There was a 13-fold variation in bean Cd and a 7-fold variation in leaf Cd between accessions despite the bioavailable Cd in the soil being uniform. There were differences in the level of partitioning into beans evident by significant variation ($P \le 0.05$) in bean Cd as a percentage of the cumulative leaf and bean Cd concentration (15–52%) between accessions. Although in general there was a higher concentration of cadmium in the testa than the cotyledon of the cocoa bean there was considerable genetic variation. These results point to the potential of using a genetic strategy to mitigate cadmium within cocoa beans either through breeding or through the use of low cadmium uptake rootstocks in grafting. The results will fuel further work into the understanding of mechanisms and genetics of cadmium uptake and partitioning in cocoa.

1. Introduction

Cadmium (Cd) is a non-essential heavy metal that is toxic to both plants and animals. In humans, low level of Cd exposure has been linked to renal dysfunction, osteoporosis and various cancers (Åkesson et al., 2014; Kazantzis, 2004; Nordburg, 2009; Nawrot et al., 2006; Nawrot

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et al., 2010). Cadmium in the soil is relatively easily transferred to food crops, and in the general non-smoking population food accounts for approximately 90% of Cd exposure (Clemens et al., 2013). Cadmium contamination of soils can occur via the weathering of rocks (Singh and Mclaughlin, 1999) and due to contamination from anthropogenic sources such as industrial activities (Clemens and Ma, 2016) as well as agricultural practices such as the application of phosphate fertilizers, sewage sludge and livestock manure (Clemens et al., 2013).

The European Food Safety Agency (EFSA) has identified chocolates as a contributor to the total human consumption of Cd and has established maximum allowable limits for Cd in cocoa based products which will become effective from January 2019 (Commission Regulation (EC), 2014). The strictest of the levels requires maximum Cd levels of 0.1 mg kg^{-1} wet weight for milk chocolate with 30% dry cocoa solids, with an increase to 0.8 mg kg^{-1} wet weight for dark chocolates containing >50% dry cocoa solids. Cocoa powder sold as a final product to the consumer will have permissible maximum levels of 0.6 mg kg^{-1}.

Studies carried out in Ecuador demonstrated Cd levels in cocoa beans ranging from 0.02 to 3.00 mg kg^{-1} with an average of 0.94 mg kg^{-1} (Chavez et al., 2015). Other studies in Latin America and the Caribbean have also demonstrated high levels of Cd in beans. Ramtahal et al. (2016) reported values ranging between 0.35 and 3.82 mg kg^{-1} in cocoa nibs produced in Trinidad and Tobago, Gramlich et al. (2017) reported mean values of 0.21 mg kg^{-1} in Bolivia and Arévalo-Gardini et al. (2017) found concentrations of over 0.8 mg kg^{-1} in 57% of cocoa bean samples from Peru. Gramlich et al. (2018) studying bean Cd levels across 6 geographical substrates in Honduras reported average values between 0.1 and 1.8 mg kg^{-1}. Zarcinas et al. (2004) reported mean levels of 0.66 mg kg^{-1} with a maximum of 1.68 mg kg^{-1} Cd in cocoa beans produced in Malaysia suggesting that the problem is not confined to the Americas.

Strategies for mitigating Cd uptake in other crops include, application of soil amendments and other cultural practices (Al-Wabel et al., 2015; Ok et al., 2011; Xu et al., 2016). Chavez et al. (2016) demonstrated that in Ecuadorian soils the availability of Cd to cocoa plants was affected by soil pH and organic matter, while Gramlich et al. (2018) reported soil pH and geology affected bean Cd concentration. This suggests that application of amendments could be effective in mitigating Cd contamination in cocoa.

Genotypic variation for Cd uptake has been demonstrated in barley, wheat, rice and leafy vegetables (Clemens and Ma, 2016); while partitioning of Cd into reproductive parts has been shown in wheat, rice, barley and sunflower (Duan et al., 2017; Harris and Taylor, 2013; Hart et al., 2006; Kashiwagi et al., 2009; Laporte et al., 2015; Sghayar et al., 2015; Williams et al., 2009; Wu et al., 2015). There have been few studies investigating the genetic variation for Cd accumulation and partitioning in cocoa (*Theobroma cacao* L.) suggesting the existence of genotypic differences. These however are limited in the number of cocoa genotypes used (<5), often evaluated under different environmental conditions (Arévalo-Gardini et al., 2017; Gramlich et al., 2017) or using seedlings of two crosses under controlled environmental conditions (Castro et al., 2015) and hence do not allow the determination of genetic variation of cadmium bioaccumulation in cocoa.

Cocoa is an understory tree species originating in tropical South America and grown for the production of cocoa beans from which a variety of confectionaries are made. Cocoa has been classified into 10 genetic groups based on phylogenetic differences and geographical origin (Motamayor et al., 2008) and into two hybrid populations, 'Trinitario' and 'Refractario' (Motilal et al., 2010; Motilal et al., 2012; Zhang et al., 2008). This study investigates the genetic variation for Cd uptake and partitioning into cocoa beans in a representative sample within the International Cocoa Genebank, Trinidad (ICGT).

2. Materials and methods

2.1. Location

The study was conducted at the ICGT, a 34-ha field cacao collection of approximately 2400 cocoa genotypes, each planted in plots of up to 16 clonal trees at a single site in Centeno, Trinidad (Location using UTM – 685617E, 1,169,849N). The ICGT is regarded as the largest and most diverse cacao collection in the public domain. The clonal trees were generated by rooted cuttings and are between 30 and 35 years of age. The soil type at the ICGT is Aquic Eutropepts and has a mean pH of 4.94. The mean annual maximum and minimum temperatures for the site were 33 °C and 22 °C, respectively. The mean annual rainfall for the site is 2000 mm.

2.2. Genotypes and sampling

One hundred accessions of cocoa representing the genetic groups viz. Amelonado, Contamana, Curaray, Iquitos, Marañón, Nacional, Nanay (Motamayor et al., 2008); as well as the two hybrid populations, Refractario and Trinitario (Motilal et al., 2013) (Table 1) were sampled during the major crop season (November to March) of 2014/15. Leaves from three genetically characterised trees per accession (35–40 leaves each) were sampled at the Interflush-2 stage (Greathouse et al., 1971). Leaves of each tree were kept separate and treated as biological replicates for analysis. Cocoa bean samples were collected from 77 of the 100 accessions as no healthy pods were available in the remaining 23 accessions. Cocoa bean samples (1–3 pods per tree) were collected from three replicate trees per accession and treated as biological replicates.

Soil core samples were collected at eight points around the drip zone of each of three sampled trees for the 100 genotypes, at a depth of 15 cm, using a stainless-steel auger (3 cm diameter). The samples collected around each tree were composited, while each of the three samples collected for each accession was kept separate and analysed as replicates.

2.3. Chemical analysis

All reagents used for sample preparation and analysis were of analytical grade and were tested in blank analysis to correct for Cd background levels. To avoid trace metal contamination, all glassware and

Table 1
List of accessions of *Theobroma cacao* investigated for leaf and bean Cd content at the International Cocoa Genebank Trinidad. Clones are grouped based on its representative genetic group.

Genetic groups	Accessions
Amelonado	AM 1/19, AM 2/61, Red Amel 1/30, ICS 60
Contamana	CRUZ 7/8, MOQ 4/6, POUND 31/A, SCA 3, SCA 5, SCA 6, SCA 11
Curaray	AGU 3339/12, AGU 3339/8, AGU 3339, EET 400, LCT EEN 31
Iquitos	CRU 72, CRU 94, IMC 38, IMC 65, IMC 67, IMC 94, IMC 105, POUND 18/A, POUND 4/A, POUND 27/C, SP 1
Marañón	PA 30, PA 34, PA 107, PA 120, PA 125, PA 132, PA 169, PA 218, PA 289, PA 296
Nacional	CRUZ 7/11, LCT EEN 68/S-1, LCT EEN73/A, MO 121, MO 80, MO 90
Nanay	AMAZ 12, AMAZ 3/2, CRU 100, NA 13, NA 26, NA 34, NA 168, NA 184, NA 191, NA 226, NA 246, NA 312, NA 719
Refractario	AM 1/53, B 12/1, B 13/3, B 5/3, CL 10/11, CL 27/50, LP 3/4, LP 4/21, SJ 2/25, SLC 19, B 9/10-30, JA 1/12, JA 4/17, JA 5/24, JA 5/35, JA 5/36, JA 5/39, LP 4/7, LV 6, LV 17, LV 20, LV 28, LX 43, LX 47, LX 50, LZ 13, SJ 1/40, SLA 30, AM 1/1, AM 1/54, AM 2/18, AM 2/32, CL 10/3, CL 15/19-7 CLM 116, LP 3/15, LP 3/40, LP 4/48
Trinitario	ICS 1, ICS 6, ICS 8, ICS 14, ICS 86, ICS 95

For more information on these cocoa accessions, visit http://www.icgd.rdg.ac.uk/.