



**A review of research on the effects of drought and temperature stress and increased CO<sub>2</sub> on *Theobroma cacao* L., and the role of genetic diversity to address climate change**

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**Disclaimer:** The objective of this document is to provide a basis of knowledge for researchers interested in working towards climate resilience in cacao. Its purpose is to allow researchers to understand the challenges that remain for breeding more tolerant varieties, and to stimulate discussion about the direction of future efforts. Views and opinions expressed here are those of the contributors and do not necessarily reflect the views and opinions of their individual institutes. In case of specific questions and/or comments, please direct them to Bioversity International.

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**Cover photo:** Cacao agroforestry system in Costa Rica. Credit: Allan Mata (CATIE)

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## EXECUTIVE SUMMARY

The global status of research on the effects of drought, temperature and elevated carbon dioxide (CO<sub>2</sub>) levels on the cacao plant, and the role of genetic diversity in producing more resilient cacao, are presented in this report. With the aim to enhance what we know about the resilience of cacao to climate change, and generate a comprehensive understanding of the questions that remain, this report highlights significant advances in published and ongoing research on drought and temperature tolerance in cacao. Most of the information about ongoing or unpublished work was obtained from personal communications and surveys involving research institutes around the globe. Organizations were selected to participate in the survey based on their presence in the relevant literature, referrals from other organizations, or personal communications from individuals attesting to their involvement in research related to drought and temperature tolerance, or increased CO<sub>2</sub> response, in cacao. A list of all the organizations contacted can be found in Appendix 10.1, while the list of questions sent out to those organizations is provided in Appendix 10.2. A brief description of the topics covered in this report is provided below.

**Climate-change models:** Models are useful tools that facilitate decision-making, answer research questions, support the mitigation of climate risks, or guide conservation, production or ecosystem management, amongst others. They also help to create a better understanding of interactions between complex traits, or scenarios that may yet occur. While modelling results can help prepare for probable climate scenarios, these results should be interpreted with caution, and any conclusions drawn should be limited to the scope and limitations of the model.

**Drought and temperature tolerance in cacao:** There are vast genetic, morphological, and physiological differences across the diverse range of known cacao cultivars. The literature highlights the potential for increased resilience of cacao through selective breeding of drought- and heat-tolerant genotypes. However, when compared to other economically important crops, the extent of investigation and publications in relation to cacao physiology and responses to drought and heat stresses is not reflective of its market value or its importance to the livelihoods of the millions of farmers cultivating cacao. Field validations and greater comparison of genetic diversity are needed to increase the scope of understanding of the behavioural and adaptation traits in cacao.

**Physiological responses of cacao:** The literature embraces four main types of physiological responses to drought and heat: osmotic adjustment, varying stomatal conductance, a range of root responses, and genetic responses.

**Osmotic adjustment:** Osmotic adjustment occurs via net solute accumulation in response to water stress. A number of cacao cultivars have been identified as being drought tolerant, based on their capacity for osmotic adjustment. In some of the cultivars, observed osmotic adjustment was a result of potassium and phosphorus accumulation in the leaf. However, the literature also indicates that a cacao plant's capacity for osmotic adjustment might be limited in terms of providing comprehensive drought tolerance, and thus also limited as a basis for selection.

**Stomatal conductance:** The ability of crops to maintain water-potential values and turgor under water-limiting conditions is an important physiological adaptation to drought. Maintenance of turgor and stable water potential values can be attained through stomatal control, deeper rooting towards additional water, and leaf rolling to avoid increased evaporative demand, among other traits. The capacity of cacao to regulate stomatal closure as a way to sustain a high relative water content, despite the drought, suggests that stomatal sensitivity is an interesting target for future efforts towards breeding for drought-tolerant



cacao, especially in seedlings. Thus, the ability to maintain high leaf water status should be a priority in the breeding of more tolerant genotypes.

**Root responses:** Allocation of carbohydrates towards the root for deeper more expansive soil proliferation is a well-known physiological and morphological response that allows many crops to escape drought. Observed differential root growth, specifically fine root growth, has demonstrated potential as an attractive drought tolerance trait to investigate in several greenhouse trials. However, given that a large proportion of the roots that branch out in field-established trees are located in the top 0.2-0.4m of soil, the probability of this trait serving as a method of escape by accessing deeper water reservoirs under field conditions might be limited. It was noted that companion planting with deeper rooting species such as *Gliricidia* appeared beneficial during times of water stress, supporting an agroforestry-system approach to managing drought stress.

**Genomic tools:** Recent advances in genomic (and other 'omic') tools and methods contribute to our understanding of underlying mechanisms for drought and temperature tolerance in cacao. Studies show that genes regulating polyamine induction or biosynthesis are commonly associated with responses to biotic and abiotic stresses, and have been shown to function in drought tolerance. Altering polyamine levels in cacao might result in enhanced tolerance to multiple stresses including drought. Further evaluation of such differences in breeding programmes is needed to understand polyamine's role in resilience.

**Genetic variation towards recombination:** Evaluations made at numerous collections around the globe suggest that enough variation exists to effectively select and breed for superior cultivars. Some cultivars have displayed up to a seven-fold difference in dry bean yield during drought stress. Canopy characteristics are also important, and materials in the collections offer the potential to breed for more photosynthetically-efficient cacao canopies. Breeding for such traits may improve the performance and sustainability of cacao under specific growing conditions. Studies have also looked at the mechanisms of trait inheritance and aimed to maximize hybridization (Santos et al. 2016). One cited study found superiority of additive effects over non-additive effects in almost all plant attributes recorded (Padi, Ofori and Arthur 2016). The combined potential of selected cacao cultivars with high heritability and genetic variation in tolerance levels recorded is promising.

**Soil mineral nutrition and drought:** There is uncertainty and ambiguity as to the right levels of mineral nutrition that should be applied to ensure healthy and balanced cacao production. Much of the primary research available was conducted over 40 years ago, with few treatments compared, and limited variables evaluated. However, based on the concept that a healthy cacao tree under balanced nutrition is more likely to be resilient to abiotic stresses, mineral nutrition as a method to mitigate the effects of climate change is a topic that is being increasingly investigated. Proposed and ongoing mineral nutrition studies on this topic, led by the Cocoa Research Institute of Ghana (CRIG) and Wageningen University and Research (WUR), have developed upon the hypothesis that mineral nutrition, particularly potassium, can play a major role in drought mitigation for cacao.

**Management and shade:** Agroforestry systems can provide income diversification and stress alleviation by reducing vulnerability to drought, especially during seedling establishment. Shade has been seen to reduce stomatal conductance, resulting in greater photosynthetic rates and enhanced survival. The data highlight the importance that crop diversification in an agroforestry system can have, not only towards the potential of reducing abiotic stress, but of also increasing total system yields.

**Interaction between drought and increased CO<sub>2</sub>:** Physiological responses of cacao to increased CO<sub>2</sub> concentrations were originally investigated by researchers at the United States Department of Agriculture

(USDA). They concluded that overall the impacts of increased concentrations would be positive. Researchers at the University of Reading have continued to evaluate in more detail cacao responses to environmental changes and rising CO<sub>2</sub> levels, in combination with decreased precipitation. Two PhD studies exploring the effects of increased CO<sub>2</sub> and drought have supported the findings of positive responses to increased CO<sub>2</sub> concentration, potentially mitigating some limitations caused by water deficit. The continuing phase of this work recently initiated (2017-2021) expands the scope of the previous research to consider resilience to high temperature, as well as the interaction between temperature and elevated CO<sub>2</sub>, and between temperature and water stress. Work at James Cook University is currently evaluating the interaction between temperature and elevated CO<sub>2</sub>.

Fundamentally, the literature compiled in this report serves as a basis to understand the questions that still remain regarding cacao's responses to abiotic stresses, highlight the resources that are available to answer them, and identify synergies and complementarities. The report also helps to identify key research questions and partners for the development of a proposal for an international/multi-institutional research programme, to be implemented over the next three to five years, as part of the Collaborative Framework for Cacao Evaluation (CFCE). Although future climatic predictions are worrisome, the genetic materials held within national and international collections offer much potential in the development of improved planting material. The objective of the report is to gather as much information as possible, so that we can aim to maximize the resilience of cacao through the discovery and use of improved planting material, in combination with improved management practices.

## ACRONYMS

BCCCA	Biscuit, Cake, Chocolate and Confectionary Association
CAOBISCO	Association of Chocolate, Biscuit and Confectionery Industries of Europe
CO <sub>2</sub>	Carbon dioxide
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza
CCI	Cocoa Coconut Institute
CENTA	Centro Nacional de Tecnología Agropecuaria y Forestal
CEPEC/CEPLAC	Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura Cacaueira
CEPROMA	Centros de Procesamiento y Mercadeo de Alimentos
CFCE	Collaborative Framework for Cacao Evaluation
CHRC	Chumphon Horticultural Research Center
CIAT	Centro Internacional de Agricultura Tropical
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRCA	Climate Impacts and Resilience in Caribbean Agriculture
CNRA	Centre National de Recherche Agronomique
CORPOICA	Corporación Colombiana de Investigación Agropecuaria
CPCRI	Central Plantation Crops Research Institute
CRA/CRUK	Cocoa Research Association Ltd./Cocoa Research (UK) Ltd
CRC/UWI	Cocoa Research Centre, University of the West Indies
CRIG	Cocoa Research Institute of Ghana
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRU	Climate Research Unit
ECA	European Cocoa Association
FCC	Federation of Cocoa Commerce
FHIA	Fundación Hondureña de Investigación Agrícola
FTA	CGIAR Research Program on Forests, Trees and Agroforestry
GCA	General combining ability
GCGRA	Ghana Cocoa Growing Research Association
GCM	Global circulation model
GHCN	Global Historical Climatology Network
GPCC	Global Precipitation Climatology Centre
ICAR	Indian Council of Agricultural Research
ICCRI	Indonesian Coffee and Cocoa Research Institute
ICQC	International Cocoa Quarantine Centre
ICRAF	World Agroforestry Centre
ICG-T	International Cocoa Genebank, Trinidad
ICT	Instituto de Cultivos Tropicales



IDIAF	Instituto Dominicano de Investigaciones Agropecuarias y Forestales
IITA	International Institute of Tropical Agriculture
INGENIC	International Group for Genetic Improvement of Cocoa
INIA	Instituto Nacional de Investigaciones Agrícolas
INIAP	Instituto Nacional de Investigaciones Agropecuarias
INRA	Institut National de la Recherche Agronomique
INTA	Instituto Nicaragüense de Tecnología Agropecuaria
IPCC	Intergovernmental Panel on Climate Change
IRAD	Institute of Agricultural Research for Development
JCU	James Cook University
Mars	Mars, Incorporated
MaxEnt	Maximum entropy
MCB	Malaysia Cocoa Board
MCCS	Mars Center for Cocoa Science
MIT	Massachusetts Institute of Technology
MMSP	Mabang Megakarya Selection Programme
PPFD	Photosynthetic photon flux density
RAPD	Random Amplified Polymorphic DNA
SAP	Super-absorbent polymer
SDM	Species distribution models
SSR	Simple sequence repeat
STCP	Sustainable Tree Crops Program
UA	Universidad de los Andes
UCV	Universidad Central de Venezuela
UESC	Universidade Estadual de Santa Cruz
UK	United Kingdom
UNAS	Universidad Nacional Agraria de la Selva
UNCR	Universidad Nacional de Costa Rica
UNSAAC	Universidad Nacional de San Antonio Abad del Cusco
UoG	University of Göttingen
UoR	University of Reading
USDA	United State Department of Agriculture
USDA-FAS	United States Department of Agriculture, Foreign Agricultural Service
USDA-TARS	United State Department of Agriculture- Tropical Agricultural Research Service
VPD	Vapour pressure deficit
WCF	World Cocoa Foundation
WUR	Wageningen University and Research

## 1 INTRODUCTION

Although there is no general agreement on how climate change has or will affect cacao, there is a wide-ranging consensus that climate instability, caused by climate change, is impacting cacao production. Meteorological data and overall model predictions suggest that significant climatic changes are likely to continue over time, with one of the main changes resulting in higher temperatures. The projected rise in temperature has been mainly attributed to the atmospheric concentration of greenhouse gases such as carbon dioxide, methane, and nitrous oxide. These gases absorb energy emitted by the earth's surface, and retain a fraction of this energy in the atmosphere (Bari et al. 2016). The imbalance between energy absorption and removal, where more energy is absorbed than released, causes temperature increases.

Within many of the research groups looking at the effects of climate change on cocoa production, there is an understanding that weather patterns will increasingly affect production and processing, and that expected prolonged dry seasons could result in increased seedling mortality, reduced productivity and tree death. In fact, there are reports that these changing weather patterns are already affecting cocoa production (Anim-Kwapong and Frimpong 2004; Eitzinger et al. 2015; Hutchins et al. 2015; Jacobi et al. 2015; Läderach et al. 2013; Nwachukwu, Ezeh and Emerole 2012; Oyekale, Bolaji and Olowa 2009; Ruf, Schroth and Doffangui 2015; Schroth et al. 2016). These observed or perceived effects are concerning given cacao's evolutionary adaptation as an understory tree in the tropical Amazon, where temperatures range from 22 degrees Celsius (°C) to 27 °C, and precipitation is consistent (Alvim and Kozlowski 1977; Motamayor et al. 2002). Given this evolutionary adaptation, cacao has developed intrinsic physiological and morphological characteristics that can make it rather susceptible to extreme weather patterns. Thus, the narrowness of the geographical range considered suitable for cacao production raises concerns about its future and its capacity to adapt to the changing environments. Nonetheless, cacao's full diversity has yet to be characterized for thresholds on increased drought, temperature or CO<sub>2</sub> tolerance, yet genetic variation has already been identified in response to environmental variables such as maximum temperatures and water deficit. Thus, the key to climate resilience should lie in exploiting the full range of cacao's genetic diversity for the selection and breeding of drought- and heat-tolerant genotypes.

The goal of this report is to present the global status of research on drought and heat tolerance and the role of cacao genetic diversity. It evaluates what the models predict will occur to climates in cacao-producing regions, what is known about cacao climatic resilience and responses, how foreseen stresses may interact with increased CO<sub>2</sub> concentrations, and what trials and evaluations are currently underway that will allow for the continued generation of novel information on cacao climate resilience. Organizations were selected to participate in a survey and interviews based on their presence in the relevant literature, referrals from other organizations, or personal communications from individuals stating their involvement in research concerning drought and temperature tolerance, or response to increased CO<sub>2</sub>, in cacao. The information gathered from those organizations that responded to the surveys and interviews has been incorporated into the main text (cited as personal communications), and summarized per institution in Appendix 10.3. A list of the questions sent out to the organizations can be found in Appendix 10.2.

## 2 CLIMATE CHANGE MODELLING AND CACAO

Models are tools that can be used to identify threats of climate change, and assist in decision making by helping to answer research questions, guide crop management practices, and mitigate climate risks, among others. They also help to better understand interactions between complex traits or scenarios, and allow evaluation of circumstances that may yet occur, such as climatic variations. With this aim, species distribution models (SDMs) along with climate change modelling exercises have been used to understand how the climate will change in cacao-producing regions, and how these changes can/will potentially affect productivity.

With 70% of global cacao production coming from West Africa, there is an urgent need to recognize how climatic variations will affect production in this region. Bari et al. (2016) observe that the Climate Research Unit (CRU), the Global Historical Climatology Network (GHCN) and the Global Precipitation Climatology Centre (GPCC) datasets all provide evidence of a drying trend in parts of West Africa. A broad review of the literature by Kotir (2011) found that Africa is already exhibiting significant climate changes. Fluctuations are evident from the variations recorded in average temperature, amount of rainfall (intensity and frequency) and prevalence of extreme temperatures, particularly along the margins of semi-arid and arid areas (Kotir 2011). These measurements are concerning given that temperature is one of the main limiting factors in cacao production, and that cacao growth and development, flowering, and fruit development are all highly dependent on temperature (Almeida and Valle 2007; Daymond and Hadley 2004, 2008). In fact, early studies cite the maximum daily temperatures at which cacao can be grown without reducing yield to be between 30 °C and 32 °C (Snoeck et al. 2010; Wood and Lass 1985). Despite this, there are cacao-growing regions in Malaysia and West Africa where temperatures can exceed 40 °C without significant negative effects. Nonetheless, concerns persist where warmer temperatures may affect cacao indirectly, via the increase of evaporative demand coupled with decreased rainfall (Anim-Kwapong and Frimpong 2004; Carr and Lockwood 2011; Läderach et al. 2013).

A 2011 study by P. Läderach (2011), aimed at predicting the impact of climate change on the suitability of cacao production in regions of Ghana and Cote d'Ivoire, gave a significantly adverse prognosis for production. The study used predicted climatic changes to forecast the suitability of current cacao-growing areas to continue producing cacao in 2030 and 2050. Their results revealed that temperatures would increase on average by 1.2 °C by 2030, and by 2.1 °C by 2050. As a result, Läderach concluded that climate suitability for cacao would decrease dramatically in the Agneby, Lagunes, Moyen-Comoe and Sud Comoe regions in Côte d'Ivoire, and also in the western region of Ghana (Läderach 2011). The study also revealed that production suitability would increase in areas of the Kwahu Plateau, between Eastern and Ashanti regions in Ghana. Using a similar but refined statistical crop suitability model, a follow-up study was conducted (Läderach et al. 2013). Similar to their 2011 study, the 2013 study used the current distribution of cacao-growing areas, along with climate change predictions from 19 global circulation models (GCMs), and an adapted maximum entropy (MaxEnt) model, to generate their predictions. The projections for Ghana were made using precipitation data from 107 stations, mean temperatures from 84 stations, and minimum and maximum temperatures from 20 stations. For Côte d'Ivoire, precipitation data were collected from 113 stations, mean temperatures from 30 stations, and minimum and maximum temperatures from 12 stations. The results are still concerning in general, citing an increase in potential evapotranspiration that is not

compensated by a change in rainfall and only partly by a slightly more favourable rainfall distribution with a shorter dry season. Although the projections are not as drastic as predicted by the earlier model, the authors predict many areas in Ghana and Côte d'Ivoire will no longer be suitable for cacao production during the 2050s, (Läderach et al. 2013). The greatest impacts are expected to be felt near the forest-savannah transition zones, with neutral or positive effects at higher elevations.

In a more recent exercise, the MaxtEnt model was combined with different climatic variables commonly considered to be critical for cacao, to evaluate the vulnerability of the West African cacao belt (Schroth et al. 2016). While many of the previous conclusions remain – i.e. that increased temperatures, specifically during the dry months, will be more limiting than low precipitation itself – Schroth et al. (2016) found scenarios that differ on the severity of expected impacts to cacao production. This is because some regions currently and naturally experience dry periods without the cacao tree being negatively affected. Enough moisture is accumulated during the wet season to sustain the tree over those dry periods. The worry is that as trees evapotranspire more in order to keep cool during the higher temperatures, the remaining moisture accumulated during the wet months will no longer be sufficient to meet the tree's water demand. Yet, the models used by Schroth et al. (2016) predict that in addition to increased evapotranspiration, the dry seasons will be shorter, and that this decrease in the length of the dry season will compensate for the higher water demand. However, soil nutritional or water retention properties of a given production system are not accounted for in the models. In many cases, the soil nutrition is so depleted that there is very low buffer capacity and low organic matter to actually retain enough moisture to support the higher water demand (Van Vliet et al. 2017). Greater understanding of how soil properties affect the interaction of these events is vital. Thus, Schroth et al. (2016) emphasize the need for more temperature-tolerant cacao genotypes and the use of shade trees to mitigate increased temperatures, as shade can potentially decrease evaporative demand but also, depending on the companion tree, enhance soil properties by increasing soil organic matter.

Even though models disagree on the severity of effects of climate change, cacao farmers, breeders and researchers seem to agree that climate change is currently affecting production, and that the combination of increased evaporative demand and reduced water availability will be of most concern (Anim-Kwapong and Frimpong 2004; Hutchins et al. 2015; Jacobi et al. 2015; Kotir 2011). A recent survey of cacao farmers in Ghana confirmed some of these predictions have already become a reality (Hutchins et al. 2015). The survey aimed to gather information on the perspectives of farmers concerning the impacts of climate change on their farms. All 13 farmer participants unanimously confirmed that weather conditions have been changing over the last 20 years. A study looking at climatic data gathered from the Ghana Meteorological Agency over a 40-year (1951-2000) period supports the notion of decreased annual precipitation. Nonetheless, according to the data not all areas are equally affected, with southwestern Ghana most affected. Records show that precipitation has decreased by up to 20% in the forest regions, and by 10% in the savannah areas (Owusu and Waylen 2009). Predictions of future patterns follow a similar trend: according to Anim-Kwapong and Frimpong (2008), cacao-producing zones of Ghana will receive increasingly less rainfall over the next 70 years (-3.1, -12.1 and -20.2 mm, by 2020, 2050 and 2080 respectively).

In Nigeria, cacao is cultivated on around 1.34 million hectares, and is the most prominent export crop in terms of production and export capacity (Nwachukwu, Ezech and Emerole 2012; Oyekale, Bolaji and

Olowa 2009). As in many other countries, significant declines in yield threaten to destabilize the country's economy. A study by Nwachukwu, Ezeh and Emerole (2012) evaluated the relative effects of climatic changes on the productivity of cacao in Nigeria by assessing climatic data for a fifty-year period (1961-2010). In their analysis, rainfall had a significant negative coefficient at < 5%, implying a reduction in rainfall over the study period. Temperature on the other hand had a significantly positive coefficient, implying increases over the study period. Thus, the records revealed that coupled with rising temperature, overall rainfall has been continually decreasing. Although the study evaluates general trends rather than specific numbers on the degree of severity, the authors voice general concern for the future of cacao cultivation. This concern is also echoed by Nigerian farmers, who state they are already feeling the impacts of climate change through delayed flowering, delayed or reduced pod development, and diminished seedling establishment. In a survey of 60 randomly selected farmers from the cacao-producing regions of Kwara State, the majority of respondents stated that the main climatic shift perceived was low rainfall (Agbongiarhuoyi et al. 2013).

Central America, another important cacao-producing region, has been impacted by climatic fluctuations. In Costa Rica, the temperature has increased from between 0.2 °C and 0.3 °C per decade since 1960, with the dry seasons becoming longer and hotter (Phillips 2015; Hutchins et al. 2015; Deheuvels et al. 2012). Projections for the 2050s and 2080s in Costa Rica indicate increases in temperature of 1-2 °C and 2-4 °C respectively, particularly for the hotter months of May to June (World Bank Group 2011). Studies in Trinidad and Tobago similarly express concern for threats to cocoa production. A survey recently showed that the greatest concern for cacao farmers in Trinidad and Tobago was drought, followed by floods, and pests and diseases (Eitzinger et al. 2015). In Jamaica, cacao is likely to be subject to small reductions in suitability, with the drier areas of the islands being more affected (Eitzinger et al. 2015). Scientists at the Bioversity Costa Rica station, in association with the World Agroforestry Centre (ICRAF), are currently working with species distribution modelling approaches, to assess the impacts of climate change on the suitability of 50 shade species widely used in cacao systems in Central America (Kaue de Sousa, personal communication, 2017). With this research, they aim to assess how changes in tree distribution (contraction or expansion) overlap with cacao areas vulnerable to climate change. The information already generated will provide insights into the potential of agroforestry adaptation options available for cacao cropping systems in Central America under climate change, and which areas hold the most potential to develop or maintain diversified cacao agroforestry systems in the region (de Sousa et al. manuscript in preparation).

While cacao suitability models generate useful information, and establish a baseline for general weather patterns, trends and impacts to production, important caveats recommend their cautious interpretation. Other important parameters must be applied to the discussion before definitive conclusions are made, with an understanding that recommendations are limited by the uncertainty of modelling exercises, and that many models are not incorporating functional components such as the availability of temperature-/drought-resistant varieties. In relation to cacao, assumptions can be made, with an understanding of the great genetic diversity of climatic thresholds, that certain clones can withstand the prevalent and predicted climate extremes. These kinds of characterization and genetic variability have yet to be incorporated into the models used to predict cacao production suitability. Climatic models that predict suitability of cropping systems should be used as a way to generate information that allows researchers, producers, farmers and industry to prepare for the future. These predictions should not be considered as definitive nor definitively alarmist. Modelling should continue

to inform the debate on how science, breeding and research can prepare for any of the predicted challenges, and how we may best mitigate the expected impacts through an extensive evaluation of the existing broad range of cacao genetic diversity and improved management practices.

Future modelling work, such as the research project proposed by Wageningen University and Research (WUR) and the University of Reading (UoR) on the effects of climate change on cacao physiology, will provide critical insights (Niels Anten and Paul Hadley, personal communications, 2017). These cacao physiology models will be instrumental in filling the gaps in knowledge and predictions resulting from sole reliance on spatial distribution models and climate modelling exercises that do not incorporate cacao genetic diversity or differential responses to stress. Only then can we start to make conclusions about cacao suitability in the coming years.



### **3 RESEARCH ON THE EFFECT OF ABIOTIC STRESS ON CACAO**

#### **3.1 Responses of cacao to increased temperatures**

Although most models cite temperature as one of the most limiting factors for future production, little research has been conducted to evaluate how higher temperatures impact production. Major effects of increased temperature have been observed on time-to-fruit ripening, fruit losses from cherelle wilt, final pod size, bean size and bean lipid content (Daymond and Hadley 2004; Daymond and Hadley 2008). In a study by Daymond and Hadley (2004), greenhouses with controlled environments were used to simulate the temperature conditions of three cacao-growing regions (Bahia, Brazil; Tafo, Ghana; and Lower Perak, Malaysia) over the course of a year, for evaluating changes in vegetative growth of three clones: SCA 6, AMAZ 15/15 and SPEC 54. Significant differences to temperature responses were observed with a higher growth rate in the warmer simulated Malaysia. In a continuing study simulating the same growing regions (Bahia, Brazil; Tafo, Ghana and Lower Perak, Malaysia), Daymond and Hadley (2008) evaluated the effects of temperature and light integral on fruit growth and development for five cacao genotypes: Amelonado, AMAZ15/15, SCA 6, SPEC 54/1 and UF 676. Fruit losses from physiological wilt (cherelle wilt), as well as fruit growth were greater at higher temperatures and differed significantly between genotypes (Daymond and Hadley 2008). Nonetheless, temperature fluctuations seemed to affect how temperature impacts growth, as reflected in the different regions of the experiment (Bahia, Brazil; Tafo, Ghana; and Lower Perak, Malaysia). In the simulated environments of Brazil and Ghana, where temperature was the main variable, temperature increases resulted in a significant decrease in the final pod size of three genotypes (Daymond and Hadley 2008). Genotype distinctions reflect genetic differences in competition for assimilates between vegetative and reproductive components. It is evident that more research is needed to fully understand how predicted increases in temperatures will affect production, and, most importantly, what diversity in tolerance and response mechanisms is out there that can better meet these challenges.

#### **3.2 Responses of cacao to drought**

##### **3.2.1 *Physiological responses***

Drought can be defined as 'a decrease in the water inputs (precipitation) into an agro/ecosystem over time that is sufficient to result in soil water deficit' (Gilbert and Medina 2016, p. 639). Many studies, the majority of which focus on seedling stage, have observed that cacao productivity, development and quality are strongly affected by the amount, distribution and duration of rainfall (Almeida and Valle 2007; Balasimha, Daniel and Bhat 1991; Moser et al. 2010). Consequently, cacao is not generally considered resilient in the face of extreme weather conditions, particularly during prolonged periods of drought (Alvim and Kozlowski 1977; Almeida and Valle 2007). Cacao's noted low tolerance to prolonged water limitation is concerning given that climate models predict reduced rainfall, and increased temperatures will become significant limitations to production in the near future, with some areas of West Africa already being affected (Agbongiarhuoyi et al. 2013; Bari et al. 2016; Hutchins et al. 2015). While there are vast genetic, morphological and physiological differences among the diverse cacao cultivars, there are key characteristic physiological trends and responses of cacao to perceived stresses. Visible symptoms under drought include premature leaf fall, yellowing of basal leaves,

wilting, small leaves, slow trunk growth and even seedling death (Carr and Lockwood 2011). Nonetheless, there is a void in the literature in relation to extensive comparisons of cacao genetic diversity, and the response and adaptation of the different cultivars to extreme conditions. Though we need to enhance our understanding, several studies have already been carried out on cacao physiology and its reaction to water deficit, temperature fluctuations, and shade management, among other factors.

This review of research covers four main types of physiological responses to drought and temperature: osmotic adjustment, varying stomatal conductance, a range of root responses, and genetic responses; these responses are detailed in the sections below.

### *3.2.1.1 Osmotic adjustment*

Osmotic adjustment is defined as the active accumulation of solutes or osmolytes in response to water limitation. It has been recognized as an important response mechanism for the drought tolerance of many crops, such as rice (Wullschleger and Oosterhuis 1991); sorghum (Tangpremsri, Fukai and Fischer 1995); maize (Apshara, Rajesh and Balasimha 2013); cabbage (Maggio et al. 2005); barley (Blum, Zhang and Nguyen 1999); and wheat (Moinuddin et al. 2005; Morgan 1992). Several studies on cacao also cite osmotic adjustment as a positive mechanism to withstand water deficit. A greenhouse trial in Brazil comparing eight cacao clones (SIAL-70, TSH-516, SPA-5, CC-10, CEPEC-519, CEPEC-84, EET-376 and SGU-54), grafted onto five-month-old seedlings of 'cacao comum' for 30 months, showed that the severity of water deficit in the different clones varied due to the capacity of some lines to induce osmotic adjustments (Almeida et al. 2002). Three clones were identified as being drought tolerant on the basis of the degree of osmotic adjustment recorded (SPA5, SIAL70 and TSH516). The observed osmotic adjustment was a result of potassium and phosphorus accumulation in the leaf.

Osmotic adjustment has also been recognized as a way to maintain turgor and higher survival rates during water deficit in field-established cacao trees. An impressive study of a shaded cacao agroforestry system in Sulawesi, Indonesia evaluated six-year-old trees over a 13-month period under imposed drought conditions to evaluate water-deficit tolerance by assessing root growth, yield, and biomass allocation (Köhler, Schwendenmann and Hölscher 2010; Moser et al. 2010; Schwendenmann et al. 2010). The imposed drought was achieved by the establishment of a large sub-canopy roof on top of the cacao trees that reduced the rainfall by 80%. The roof trapped and then channelled the rain outside of the designated dry experimental plots. Surprisingly, at the end of the drought period, treatment differences were not as significant as expected. While root water potentials decreased and yield declined by 10%, in the water-deficit treatments, no significant differences in the root biomass or primary production of roots were observed between the well-watered and water-deficit treatments. According to the authors, the observed decline in root water potentials, while partly attributed to tissue dehydration, may also suggest that cacao can achieve significant osmotic adjustment within its roots. The localized osmotic adjustment response in the root tissue is hypothesized to have aided in the stabilization of plant water status as well as sustained leaf, stem and root growth (Moser et al. 2010). Other reasons cited for the lack of significant treatment effects include mitigation of stress by overhead canopy shading and reduced evapotranspiration, and access to additional water despite the authors not observing deeper root growth (Köhler, Schwendenmann and Hölscher 2010; Moser et al. 2010).

In an irrigated field trial at an experimental station of the Instituto Nacional de Investigaciones Agrícolas (INIA) in Merida, Venezuela, four-year-old trees were evaluated for drought tolerance (specific genotypes were not mentioned). For the water-deficit treatment, trees were left for 3, 12, and 25 days without irrigation. With just an average precipitation of 570 mm, this Venezuelan region receives much less precipitation than that which is regarded as adequate for cacao cultivation. According to Alvim and Kozłowski (1977) less than 1,200 mm a year can result in soil-water deficit and reduce growth and yield. Such climatic conditions make this location ideal for water-deficit trials. At the end of the study, tolerant trees had lower leaf-transpiration rates and considerably lower stomatal conductance as compared with control plants, and also showed osmotic adjustment responses (Rada et al. 2005). Osmotic adjustment and sustained leaf turgor was observed in the initial 12 days after water removal. Nonetheless, the trees were not able to sustain this response over a period of 25 days without irrigation (Rada et al. 2005). These results show that the ability of osmotic adjustment to provide the evaluated cacao genotypes with tolerance to water deficit, might still be limited.

Another study carried out by the Universidad Central de Venezuela (UCV) and the Universidad de los Andes (UA) in Venezuela, observed the drought tolerance of Criollo-type cacao trees that had been recently established under an agroforestry system (Araque et al. 2012). The Criollo cultivars evaluated in this system were Porcelana, Guasare, Porcelana and Criollo merideño. The trees were assessed over the course of two dry periods: 7-13 months after planting. In general, stomatal conductance decreased in all genotypes by 60%, resulting in a 73% decrease in photosynthetic activity, with no significant differences in water potential. However, the observed variation in the level of osmotic adjustment achieved between the genotypes evaluated was enough to categorize some as more tolerant than others (Araque et al. 2012). Thus, while the authors observed differential osmotic adjustment, the response was not enough to cause differences in stomatal conductance or photosynthesis.

There is conflicting evidence about the capacity of osmotic adjustment as a drought tolerance response, and its use as a selection trait to indicate tolerance, and thus its potential use for breeding and selecting resilient cultivars. In other shade-adapted crops like coffee, osmotic adjustment is minimal, giving the perception that it might be similar for cacao, and therefore may not be the best tolerance strategy for cacao (DaMatta 2004). More research to better understand the potential of osmotic adjustments in cacao as a method to enhance water-deficit tolerance is needed to fully understand and exploit its potential. However, none of the surveyed institutions stated an interest or current work in osmotic adjustment.

#### 3.2.1.2 *Stomatal conductance*

Stomatal conductance measurements estimate the rate of gas exchange (i.e. CO<sub>2</sub> uptake) and transpiration (i.e. water loss) through the leaf stomata, as determined by the degree of stomatal aperture and therefore the physical resistances to the movement of gases between the air and the interior of the leaf (Pietragalla and Pask 2012). The ability of crops to maintain water potential values and turgor under water-limiting conditions is an important physiological adaptation towards periods of reduced water availability. When plants are exposed to increased evaporative demand, turgor and stable water potential values can be attained through stomatal control, deeper rooting to reach additional water, and leaf rolling among other traits (Boyer 1996; Cowan and Farquhar 1977; Gilbert

and Medina 2016; Sinclair et al. 2010). This is also true of cacao. Early studies by Nunes (1967) compared drought tolerance of three potted cacao genotypes known in Sao Thomé as 'Laranja Amarelo' (LA), 'Amelonado Vermelho' (AV), and 'Amelonado Amarelo' (AA). The variations in stress levels detected were attributed to differences in stomatal response and transpiration rates between the genotypes. Cultivars with greater stomatal sensitivity were able to better regulate water loss. These results were consistent with Balasimha et al. (1988), who concluded that effective stomatal regulation is a key drought tolerance response of cacao that can result in decreased transpirational water loss. In fact, the stomatal opening in cacao has been observed to be very sensitive to water deficit and relative humidity, with proven genetic variation in the level of sensitivity (Acheampong, Hadley and Daymond 2013; Acheampong et al. 2015; Gomes, Kozlowski and Reich 1987). Several studies have observed correlations between stomatal closure and decreased water potentials, or increased evaporative demand (Balasimha, Daniel and Bhat 1991; Balasimha et al. 1988; Baligar et al. 2008)

In a more recent field study at the regional station of the Central Plantation Crops Research Institute (CPCRI), in Vittal, India, eleven three-year-old cacao genotypes from five countries were evaluated under drought conditions: Santa Cruz-1, -9, -4 (Colombia); Rio Branco-33/3 (Brazil); Pound-16/A, -7/B (Peru); Rosario Izupa Marico-41, -189 (Costa Rica); and Bolivar-12/2, Javilla-1/19, Amazon-15 (Ecuador). Differences in both treatment and genotype were observed (Apshara, Rajesh and Balasimha 2013). While all the accessions showed a general decrease in the photosynthetic parameters, three accessions presented greater resilience to water deficit by reducing transpirational water loss through greater stomatal sensitivity and induced stomatal closure.

Almeida, Tezara and Herrera (2016) also observed variations in stomatal sensitivity in a Venezuelan field trial run by UCV. The study compared both seedlings and established trees of four cacao varieties subjected to drought. The genotypes evaluated were 415, a hybrid with Forastero characteristics selected for its high productivity and extensive use in commercial plantations around the area of study; 439, a hybrid of Criollo and Forastero, selected for an incidence of *Phytophthora palmivora* disease lower than 25%; 443, a hybrid of a modern Criollo and a Forastero and resistant to brown spot; and 447, a combination of hybrid and Forastero with an incidence of brown spot greater than 25% but with a high yield. Measurements were taken between November 2012, February 2013 and May 2014, during the rainy and wet seasons, in mature fully expanded leaves of 4-6 seven-year-old adult trees of each clone, similar in foliage and height. The seedlings were grown in the greenhouse, and subjected to imposed water deficit. In the greenhouse trial, stomatal closure was identified as an effective mechanism to preserve leaf-water status, indicated by the maintenance of relative water content. Lower stomatal conductance was also observed in the field, but water potentials of all the clones substantially decreased as the dry period progressed. The ability of the plants to regulate stomatal closure to sustain a relative water content, despite the drought, suggests that stomatal sensitivity is an interesting target for future tolerance breeding efforts, especially in seedlings. The authors therefore suggest that high leaf-water status, rather than optimizing water use through water use efficiency, should be the priority in breeding for more tolerant genotypes (Almeida, Tezara and Herrera 2016).

In Nigeria, a greenhouse seedling trial aimed at seedling establishment was carried out by the Cocoa Research Institute of Nigeria (CRIN), in collaboration with the University of Ibadan (UI). In the trial, the growth and responses of four of the most popular clones in the area (F3 Amazon, T1, T7 and

Amelonado) were evaluated under four different irrigation treatments: daily, three-day, five-day and seven-day intervals (Ayeboyin and Akinrinde 2016). Differential responses to the imposed drought were found between the clones. As has been observed in many species, the leaf area, stomatal conductance and photosynthetic rates recorded had linear and positive relationships with water status. Above all, the data from this trial showed that frequency of irrigation rather than amount was most important for seedling establishment. This is also important, as it gives another perspective: it's not only the amount of precipitation that should be taken into consideration, but it is the dispersion of this precipitation throughout the wet season that can really impact production and establishment.

Based on these studies, we can conclude that there is diversity in terms of stomatal sensitivity, and that differences in the degree of response can lead to differences in resilience during times of water deficit or increased temperature. With more conclusive results, stomatal conductance appears to show greater potential as a resilience trait than osmotic adjustment.

#### 3.2.1.3 *Water potential*

Like in many crops, cacao stem and leaf water potentials are used as indicators of plant water status. An earlier greenhouse study by Joly and Hahn (1989) evaluated the water potential responses of open-pollinated seedlings of three cacao cultivars. The cultivars included two of the Amazonian types (EET-399 and EET-400) and one hybrid Trinitario type (UF-613). During this study, the net photosynthesis of the seedlings started to decline in response to water deficit once the leaf water potential fell below about -0.8 to -1.0 megapascals (MPa). Comparably, another greenhouse study of open-pollinated seedlings observed the distribution of carbon-14 (<sup>14</sup>C)-labelled assimilates during water deficit. The data showed that moderate stress occurs when leaf water potential reaches values of -0.8 to -1.2 MPa, and that severe stress occurs below water potentials of -1.76 MPa (Deng, Joly and Hahn 1990).

In a field study carried out at CPCRI, Balasimha, Daniel and Bhat (1991) reported that tolerant cacao cultivars were able to maintain higher water potential values during midday hours. They compared eight genotypes that were considered drought tolerant (NC 23, NC 29, NC 31, NC 39, NC 42 and Amel x Na 33) with eight genotypes considered susceptible to drought (NC 24, NC 30, NC 52, NC 55, Re-daxil, PA7 x Na 32 and Amel x Na 32) under water-limiting conditions. Those that maintained higher midday water potential values, even under drought, were considered the most tolerant. The results of this field trial were further supported by a recent study comparing 52 genotypes imported from the International Cocoa Quarantine Centre (ICQC) at the UoR (Balasimha, Apshara and Jose 2013). The results of this trial indicated that the genotypes showing higher water potential can be considered as drought tolerant. Thus, it can be agreed that water potential levels are stable indicators of resilience, especially when a particular genotype is able to maintain high levels of water potential despite severe water limitations.

#### 3.2.1.4 *In the 'pipeline'*

New information on the physiological responses of cacao to water deficit is still being generated. Many of the organizations surveyed as part of the process of compiling information on ongoing or unpublished work related to abiotic stress, indicated a focus on different aspects of cacao physiology. Several research institutes are looking at gas exchange, specifically stomatal conductance, as a candidate trait for selection during times of stress. Among the institutions that are currently working on

stomatal conductance as one of the traits in drought tolerance trials are the Instituto Nacional de Investigaciones Agropecuarias (INIAP) in Ecuador; Instituto de Cultivos Tropicales (ICT) in Peru; the UoR in the UK; Cooks University in Australia (CU); the International Institute of Tropical Agriculture (IITA) in Nigeria; CPCRI in India; the Indonesian Coffee and Cocoa Research Institute (ICCRI) in Indonesia; and the Cocoa Research Institute of Ghana (CRIG). In a collaborative effort with the UoR, some of the accessions in the International Cocoa Genebank, Trinidad (ICG-T) held by the Cocoa Research Centre/University of West Indies (CRC/UWI), have been screened for genetic variation of physiological traits, particularly gas exchange, as a way to evaluate and exploit available variation in tolerance traits.

### **3.2.2 Root responses**

The root system is one of the most sensitive and responsive tissues of a plant, and allocation of carbohydrates to increase root proliferation typically allows for access to deeper water reservoirs during periods of water deficit, escaping drought. Several studies mention increased root mass as being a possible trait for drought tolerance in cacao. Deng, Joly and Hahn (1990) observed in water-stressed cacao a reduction in the amount of  $^{14}\text{C}$ -labelled assimilates exported to sink leaves and to expanding flush leaves, but an increase in  $^{14}\text{C}$ -labelled assimilates allocated to roots. This behaviour was recognized as a potential response to water stress that can allow roots to access deeper water reservoirs when water is limited.

Correspondingly, in response to drought, Santos et al. (2016) evaluated numerous plant morphological characteristics including the root biomass of 21 progenies from hand-pollinated crosses in a diallel scheme of seven cacao accessions (SCA-6, Catongo, Mocorongo, Pucala, IMC-67, TSH-1188 and RB-40). The progeny were grown under greenhouse conditions, and evaluated 12 months after sowing. The observed differential root growth, specifically fine root growth, showed potential as an interesting drought tolerance trait to investigate (Santos et al. 2016). These observations are interesting as earlier studies had observed differences in drought tolerance between the parental lines (SCA-6, Catongo, Mocorongo, Pucala, IMC-67, TSH-1188 and RB-40), showing heterozygosity between the resulting progenies (Santos et al. 2014).

Given the motivating results on previous cacao root physiological responses, root physiology is continually being evaluated in emerging and ongoing trials as a possible mechanism for drought tolerance. The ICT in Peru was one of the institutes surveyed that indicated root growth and architecture as playing an important role in their current evaluations (Enrique Arévalo, personal communication, 2017). Rhizotrons are being used at the CRC/UWI, Trinidad, where evaluations are currently looking at root traits in seedlings grown in greenhouses, to profile quantitative root traits under optimum and water-deficit conditions. Similarly, at the Universidade Estadual de Santa Cruz (UESC), in Bahia, Brazil, root evaluations are also being carried out as part of drought tolerance trials. Researchers are collecting data on the chemical composition, starch content, total soluble sugars and reducers in leaves and roots, as well as on osmoregulation (leaves and roots) and root architecture, to further assess the root's role in drought tolerance in cacao (Alex Alan Furtado de Almeida, personal communication, 2017). IITA in Nigeria is also looking at root architecture to increase drought tolerance; the work is still ongoing (Ranjana Bhattacharjee, personal communication, 2017).



Despite these studies, it must be noted that although cacao has relatively deep taproots, the depth from where water can be taken up depends on the length of the taproot and its lateral growths (Mommer 1999). The taproot itself is not characterized by its potential to absorb water, rather it is the lateral roots that branch out of the taproot that are responsible for water absorption. An agroforestry study of cacao with *Gliricidia sepium* (Gliricidia, Madrecacao, Madero-negro) assessed differential root growth, yield, and biomass allocation, with results truly representative of those expected in farmers' fields (Moser et al. 2010; Köhler, Schwendenmann and Hölscher 2010; Schwendenmann et al. 2010). *Gliricidia*, originating from the Central American dry forest, scavenges for its nutrition and water in deeper soil, and consequently is a widely used tree species in cacao cropping systems for both its nitrogen fixing and shade supply. As observed by Köhler, Schwendenmann and Hölscher (2010), *Gliricidia* appeared to be a good companion tree even in prolonged periods of water stress, as the cacao trees withdrew water mainly from the top portions of the soil profile, with more than 80% of the fine root biomass located in the top 40 cm of soil. The data do not entirely support the case for root proliferation as a method of escape; no treatment differences were observed in the root mass, found mostly in the upper profile. Thus, it appears that cacao and *Gliricidia* have complementary ecophysiological responses, and competition between the two species for water resources during long periods with little precipitation is low or in favour of cacao (Köhler, Schwendenmann and Hölscher 2010). Therefore, while shallow-rooted cacao may not have as much potential to develop deeper rooting for soil water extraction, such characteristics might serve for the design and planning of agroforestry systems that mitigate water deficit via other microclimatic services, such as shade.

#### 3.2.2.1 Rootstocks

It is interesting to note that several research institutions mentioned, in their responses to the survey, carrying out evaluation trials on rootstocks. However, limited information was provided on the specific rootstocks and the methods used for selection. Using tolerant rootstocks for different pressures is a common practice in many fruit tree crops (Atkinson et al. 1998; Bhusal, Mizutani and Laban Rutto 2002; Isaakidis et al. 2004). It is important to fully understand the potential of this practice for drought tolerance in cacao. A PhD study on the effect of rootstock on the genotypic drought tolerance ability of cacao was carried out in Nigeria to investigate the significance of factors such as rootstock/grafted variety interactions, and the effect on the physiological performance of the grafted variety during establishment and early growth, especially under drought conditions (Ayegboyin 2012). The data generated in the study reflected that the effect of rootstock was more pronounced for some clones than others, but that it also varied across seasons. Thus, it was concluded that since rootstocks affect the performance of the cacao plants, there is the potential for rootstock selection to be used for improving establishment and early growth rates of cacao plants, especially during the dry season in Nigeria.

At UESC and ICCRI, new studies are looking at the potential of using rootstocks selected for drought tolerance, to evaluate their potential to confer tolerance to the scion (or grafted genotype). At the CPCRI in India, several rootstocks have already been selected with this objective (Elain S. Apsara, personal communication, 2017). Similar evaluations have been done at INIAP, Ecuador, where rootstock evaluations were first carried out in the greenhouse, and then the rootstocks were established in a field trial (Ramón Jaimez, personal communication, 2017). Based on the results of the studies thus far, it appears that there is potential to increase resilience through the use of specific

rootstocks. The information that will be generated by ongoing trials will help add to the discussion regarding the interactions between shade trees and cacao, and the trade-offs that could result from those interactions; and it will shed light on the potential genetic diversity that exists in cacao to exploit the dynamics of root architecture as a way to generate more drought-resilient cultivars.

### **3.2.3 Genetic responses**

Given the technological advances in genetic tools and methods, evaluating genetic responses to stresses has become a standard way to understand plant metabolisms and plant physiology. Such is the case with cacao responses to abiotic and biotic stresses. Specifically, there is an interest in the genes that regulate polyamine induction or biosynthesis in cacao towards drought resistance. Polyamines have been commonly associated with responses to biotic and abiotic stresses, and have been shown to function in drought tolerance (Bouchereau et al. 1999; González de Mejía et al. 2003). Bae et al. (2008) evaluated the constitutive and drought inducible expression patterns of genes encoding enzymes involved in polyamine biosynthesis of two-month-old cacao seedlings. The responses correspond with what has been observed with other crop species: genes encoding enzymes are induced with the onset of drought and correlated with changes in stomatal conductance, photosynthesis, and leaf water potential. Thus, Bae et al. (2008) suggest altering polyamine levels in cacao might result in enhanced tolerance to multiple stresses including drought. Similar results were found in six-month-old seedlings of 36 cacao genotypes, exposed to well-watered and water-deficit conditions (Santos et al. 2014). This 2014 study evaluated the effects of water deficit on growth, chemical composition and oxidative stress. For the genotypes considered less tolerant (CC-40, C. SUL-4 and SIC-2), drought induced the candidate genes involved in the Abscisic acid-dependent pathway and protein biosynthesis of photosystem II, while in the majority of the more tolerant genotypes (MA-15, MO-20, and PA-13), there was repression of these genes.

With advances in genomics, analyses and tools are becoming more and more available around the world, and there seems to be greater interest and acceptability in incorporating those methods into cacao research. Some of the surveyed institutions that have mentioned working on incorporating genomic tools are: CRC/UWI; CPCRI; Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura Cacaueira (CEPEC/CEPLAC); CRIG; ICCRI; UESC; and the United States Department of Agriculture (USDA). The IITA stated that new research will employ the use of the genome editing technology CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) to address specific physiological traits of interest towards the development of drought-resistant clones (Ranjana Bhattacharjee, personal communication, 2017). Excitingly, in the near future, all the results from current and past experiments at CRC/UWI will be combined – phenotypic data (above and below ground) and genotypic data – in order to identify candidate genes for marker-assisted selection (Aidan Farrell, personal communication, 2017). However, this is an area of investigation and tool application that in cacao research is lagging behind compared to other important crops, and could be improved.

Many genomic tools are essential for efficiently, accurately and representatively selecting base populations that display genotypic variability, as well genetic heritability. As these tools become more available, they should increasingly support and complement cacao research efforts, not just in drought and temperature tolerance trials, but in breeding efforts overall.

## 4 REPORTED GENETIC DIVERSITY AVAILABLE IN COLLECTIONS

### 4.1 Collections that have characterized accessions

Understanding the potential to develop improved planting materials that can better meet anticipated challenges is important for developing resistant genotypes. Maximizing hybridization efforts has been the key for breeding improved varieties of many crops, and this approach can be used for cacao to create resilient and productive genotypes for sustainable and cost-effective cocoa production. Although cacao germplasm collections are essential in providing the basis for diverse genetic material, much of the diversity maintained in these collections is underused and/or at risk from lack of funding and support. One important step to increase the use of the genetic diversity that is already available and ready for use, is the characterization and evaluation of key traits for selection in breeding programmes.

There are several cacao collections around the world managing cacao diversity. Currently, there are only two international collections: ICG-T, managed by the CRC/UWI, and located in Trinidad and Tobago; and the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Costa Rica. These two international collections have signed an international agreement to maintain global collections of cacao genetic resources for the long term, and make them available to any bona fide user. In addition to the two international collections, the ICQC at the University of Reading in the UK allows for the safe transfer of germplasm around the world. The collections at CATIE and CRC/UWI have morphologically categorized many of their genetic materials.

For the CRC/UWI collection, molecular characterization of 600 accessions was conducted between 1994 and 2001 using Random Amplified Polymorphic DNA (RAPD) markers , and since 2001 microsatellite analysis – simple sequence repeats (SSR) – has been used for continued categorization and to assess the level of mislabelling of the remaining clones (Motilal and Butler 2003; Motilal et al. 2011; Motilal et al. 2012; Tondje et al. 2007). The objective of this characterization work has been to identify cacao types that are over- or under-represented in the collection, to assess the degree of homogeneity within accession groups, and to assess the genetic distances between them. Recently, physiological evaluations, photosynthetic rates and stomatal conductance, have been measured for some of the ICG-T accessions to assess the level of variation in such traits (Fiona Lahive, personal communication, 2017).

Likewise, the collection at CATIE has been morphologically and agronomically characterized (Engels 1981; Phillips-Mora and Enríquez 1988). This information is available for breeders in a published catalogue highlighting their top varieties (Phillips-Mora et al. 2013). More recently, characterization to confirm whether genotypes are true-to-type with respect to their morpho-geographic group is underway (Allan Mata, personal communication, 2017). Verification that all trees within a replicate (3/clone) are true-to-type has also been completed for a percentage of the collection. To date, the clonal groups PMCT and UF have been molecularly and morphologically characterized (Mata-Quirós et al. in press). Evaluations made at both CATIE and CRC/UWI suggest that enough variation exists in the collection to allow for the selection of a wide range of tolerance to abiotic and biotic stresses (Wilbert Phillips, personal communication, 2017).

Similarly, several studies have assessed the level of heterozygosity and allelic diversity present in numerous clonal gardens. In the Dominican Republic, Boza et al. (2013) identified significant genetic diversity at the collection in the Mata Larga research station of the Instituto Dominicano de Investigaciones Agropecuarias y Forestales (IDIAF). The authors confirm that there is considerable genetic variation in the collection that can be exploited in local and international breeding (Boza et al. 2013). Similarly, at the Tropical Agricultural Research Service (TARS) station of the USDA in Mayaguez, Puerto Rico, all 924 trees in the cacao collection were fingerprinted using a high throughput genotyping system with 15 microsatellite loci (Irish et al. 2010). While errors in labelling and duplicated material were identified, the material evaluated holds vast potential to select and breed for superior cultivars. Similarly, a study in Ghana evaluated the hybridization potential of 116 clones in their collection of introduced cacao clones by making associations of vigour, yield and their component traits. The data confirmed that even with a narrow genetic base in the country, there was considerable genetic variation present for yield. The authors are confident that yield increase can be achieved by developing hybrids from some of the best clones evaluated (Ofori et al. 2016).

#### **4.2 Trait variation trials**

Detailed evaluation of variation has been carried out for several traits including yield, yield components, gas exchange and others. In a collaborative trial between the Mars Center for Cocoa Science (MCCS) in Brazil, and the UoR, the canopy characteristics of ten clonally propagated cultivars were measured over a 14-month period. The relationship between leaf area index and fractional radiation interception was evaluated to estimate the scope of variation that exists for crop improvement. The observed clonal differences in the relationship between leaf area index and fractional light interception implied that differences in canopy architecture are present. These results demonstrated the potential to breed for more photosynthetically efficient cacao canopies (Daymond et al. 2002). Similarly, in another joint effort by the MCCS and the UoR, yield components and biomass partitioning of seven-year-old clones (CC-10; CP-519; CP-82; EEG-29; EET-62; ICS-9; SGU-54; TSH-56 and TSH-1188) were evaluated in a field trial in Brazil. Significant differences in dry bean yield were recorded between genotypes, with values ranging from the equivalent of 200 to 1,389 kilogrammes per hectare ( $\text{kg ha}^{-1}$ ). Significant genotypic variation in yield efficiency was also identified (Daymond et al. 2002). The contribution from beans to total pod weight varied between 32.0% (CP-82) and 44.5% (ICS-9) of the pod biomass. These results demonstrate that along with canopy architectural characteristics, there is potential to select for differences in yield and yield components of superior cultivars.

Seedling establishment is a critical production constraint of many cacao-producing countries in Africa, where planting is typically carried out in June, and the dry season starts just five months later in November. In many cases, the five-month-old seedlings are not yet established enough to withstand a prolonged or severe dry period. Thus, numerous trials have evaluated drought tolerance with a focus on seedling establishment (Frimpong et al. 1999; Ofori et al. 2014; Padi et al. 2013). Ofori et al. (2014) evaluated the drought response of cacao seedlings in the 2012/13 major dry season, under shade and full-sun locations in Ghana (CRIG), using six-month-old cacao seedlings from thirty-eight genotypes resulting from diallel (incomplete) and factorial crosses (23 and 15 respectively). The results showed that shade was an important component for seedling survival by possibly mitigating the effects of drought. However, for areas with no shade they recommended the top performers: A1/197 x

SCA9; AMAZ15/15 x PA13; Pound7 x PA7/808; and A1/197 x T60/887. A genetic analysis of the selected lines identified considerable genetic variation for traits related to drought resistance. Most importantly, the data suggest that while many of the responses, including stomatal conductance, were induced by environmental factors, inherent genetic induction was also responsible for the differential responses (Ofori et al. 2014). These results show promise for exploiting such differences in breeding programmes.

While evaluating the level of genetic variation and relationship between stem growth, seedling survival percentage, vigour and chlorophyll content of the same thirty-eight progenies, the authors found moderate to high positive phenotypic correlations ( $r = 0.37-0.69$ ) between traits. These results serve as an indicator that direct selection of one of the traits evaluated can give a favourable response to the other. Most importantly, results from the study show that there is a considerable genetic variation for the evaluated traits related to drought resistance (survival, stem growth and vigour) that is mainly due to additive variance. The results also showed that even though trait expression was influenced by the environment, trait expression was also partly influenced by endogenous factors. Thus, the authors reiterate that the best clones – PA 13, PA7/808 and AMAZ 15/15 – could be used to develop drought-tolerant cacao varieties through recurrent selection.

Morpho-physiological traits for drought tolerance were evaluated in a recent study at CPCRI to identify the potential of certain traits to be used as a way to accelerate breeding during early screening (Kacou et al. 2016). Ten cacao hybrids – VTLCP-22, VTLCP-11, VTLCP-24, VTLCP-25, VTLCP-26, VTLCP-29, VTLCP-27, VTLCP-28, VTLCH-4 and VTLCH-3 – were used in this potted greenhouse study. Leaf water potential, net photosynthesis, stomatal conductance, chlorophyll fluorescence and transpiration rate data were recorded. Kacou et al. (2016) observed genotypic variation of gas exchange, photosynthesis and photochemical activities, concluding that genetic variation for those traits is present. Thus, the authors suggest breeding for maintenance of water status, gas exchange and photochemical activities, may improve the performance and sustainability of cacao under specific growing conditions (Kacou et al. 2016).

### **4.3 Heritability**

Knowledge of the phenotypic characteristics and performance of the genotypes is not the only important aspect for breeding. Knowledge on trait inheritance is essential to guide effective breeding programmes. Santos et al. (2016) evaluated the combining ability of several genotypes already preselected for drought tolerance at CEPEC/CEPLAC in Brazil (Santos et al. 2016). In a greenhouse study, cultivars were exposed to well-watered and water-deficient conditions. Water deficit was imposed twelve months after sowing by removing irrigation for 60 days. The study found superiority of additive effects over non-additive effects in almost all the traits recorded. The combining potential of selected cacao genotypes with high heritability and genetic variation in tolerance levels recorded is promising. Thus, the study established the potential of the evaluated parental lines for future breeding trials (Santos et al. 2016). Drought tolerance heritability analysis is still part of ongoing trials. CEPEC/CEPLAC, in collaboration with the UESC, are evaluating drought tolerance heritability as part of two master's thesis projects (Alex Alan Furtado de Almeida, personal communication, 2017). The goal is to estimate genetic variance and heritability associated with specific traits and to identify possible correlations between field and greenhouse evaluations, drought vs. yield components, resistance, etc.

In another heritability study in Ghana, 64 cacao varieties belonging to various genetic groups were grown in two climatically contrasting locations and evaluated for seedling growth, survival and early yield, and also for female and male general combining ability (GCA). Genotype PA 7 was found to be the best female for survival under unfavourable conditions, whereas genotype CRG 1019/101 (male) combined with T60/887 to produce the varieties with the highest vigour, seedling survival and highest early yields (Padi, Ofori and Arthur 2016). Non-additive genetic effects were much larger than the corresponding additive variance components for all traits at each of the two locations (Padi, Ofori and Arthur, 2016). Heritability for yield efficiency was moderate ( $h^2 = 0.5 \pm 0.23$ ).



## **5 MANAGEMENT PRACTICES**

### **5.1 Mineral nutrition and drought mitigation**

The purpose of fertilizing should be to feed the crop by replenishing exported or leached nutrients, in order to achieve the optimum balance for a sustainable production (Snoeck et al. 2013). Yet, there is uncertainty and ambiguity as to the right levels of mineral nutrition that should be applied to achieve a healthy and balanced cacao production. Much of the primary research available was conducted over 40 years ago, with few treatments compared, and limited variables evaluated (Van Vliet et al. 2017). As a result, farmers are generally not well informed regarding correct fertilizer or soil health practices, and many researchers remain ambiguous as to the right levels of nutrition. However, based on the concept that a healthy cacao tree under balanced nutrition is more likely to be resilient to abiotic stresses, mineral nutrition as a method to mitigate the effects of climate change is a topic that is increasingly being investigated, and still needs greater exploration and understanding.

One leading soil mineral amendment highlighted for its potential to mitigate the effect of drought in cacao orchards is potassium. Interactions between potassium nutrition and drought stress have been observed in other crops – e.g. sorghum (Asgharipour and Heidari 2011); cassava (Ezui et al. 2017); olive (Erel et al. 2014); and highland banana (Taulya 2013) – and could be a management opportunity towards drought resilience adaptability in cacao. Aside from depending on potassium to regulate intrinsic osmotic functions, cacao has a specifically high demand for potassium, particularly for pod structure, and so it could benefit from supplemental potassium. Therefore, the best period to apply potassium is thought to be during pod set and development (Almeida and Valle 2007). In cacao, potassium (K) is typically supplied in the form of potassium chloride (KCl). However, more research is needed to quantify the right concentrations and find ways to make recommendations that are location specific, as when applied in excess potassium shortens leaf life and accelerates leaf fall (Snoeck et al. 2013). Ongoing and proposed mineral nutrition studies on this topic, led by CRIG and WUR, have been developing on the hypothesis that mineral nutrition, particularly potassium, can play a major role in drought mitigation in cacao (Francis Padi and Ken Giller, personal communications, 2017).

Future studies should continue with an understanding of the reasons why many farmers do not currently use fertilizer on their fields. According to Ruf (2009), there are three main reasons: (1) farmers are not well informed about the correct use of fertilizers; (2) access to chemical fertilizers is difficult; and (3) chemical fertilizers are costly. Understanding and finding ways to overcome these limitations should be one of the first steps to ensure successful implementation of the guidelines and recommendations that would result from future research.

### **5.2 Shade**

Agroforestry systems have for many years been the traditional method of planting cacao. These systems, aside from providing income diversification, also provide stress alleviating services (Jacobi et al. 2015; Verchot et al. 2007; Tschardt et al. 2010; Beer et al. 1998). In coffee, shade trees protect the plants from drought by reducing the evaporative transpiration demand, and increasing the infiltration capacity of the soil (Lin 2007). Shade can affect cacao stomatal conductance, ameliorating the effects of drought especially during seedling establishment. Such interaction was observed by Frimpong et al. (1999), when they evaluated the seedling establishment responses of 26 cacao

families in bare, denuded areas. It was noted that shade increased seedling vigour and field survival; three families –T60/887 x Pound 7; T60/887 x Pound 10; and T60/887 x Pound 15 – were identified as being the most drought tolerant. In a more recent study, the authors observed that shade, through reduced evaporative demand, decreased the negative effect of water deficit on stomatal conductance, resulting in greater photosynthetic rates and enhanced survival (Acheampong et al. 2015).

Dynamic agroforestry systems are the concept behind what is thought to be a more sustainable approach for cocoa production. These systems are established based on the knowledge of succession and structure of the natural ecosystem, with two main features: '(i) high planting densities and diversity, stratification, and a high energy flow usually without the use of external inputs; and (ii) management practices such as different types of pruning interventions, e.g. rehabilitation, formative and maintenance pruning, selective weeding or grafting, and selection of healthy, productive planting material' (Christian et al. 2013, p. 128-129). Christian et al. (2013) observed that although monocultures achieved higher levels of cacao dry bean yields, total system yields of monocultures could not reach those of the three agroforestry systems evaluated. Thus, these results heighten the importance that crop diversification in an agroforestry system can have, not only towards decreasing the impacts of abiotic stress, but also towards increasing total system yields.

However, these types of production systems are in decline. Aided in the 1960s by a push from government to maximize short-term yields (Clough, Faust and Tschardtke 2009; Ruf, Schroth and Doffangui 2015), such practices have been shifting away towards more intensive monoculture production. These monoculture systems tend to be aided by high inputs, with studies highlighting a strong interaction between fertilization and shade, showing that cacao without shading, but with fertilizer inputs, can produce high yields (Snoeck et al. 2013). Still, some shaded agroforestry systems that use the leguminous tree *Gliricidia* as companion, have been seen to sustain a productivity of about 700 kg of cacao beans per hectare without fertilization (Bastide et al. 2007).

Although shade has been found to reduce stress during periods of high temperature and decreased water availability with limited inputs, the available literature is not always clear as to the appropriate levels of shade or the most favourable tree companion species in specific regions or countries. As such, greater long-term research is needed to truly understand the most effective way to structure these systems.

## 6 INCREASED CO<sub>2</sub> AND ENVIRONMENTAL INTERACTIONS

Atmospheric CO<sub>2</sub> levels are continuing to rise, and along with this other climate variables are also expected to be altered. Initial work was conducted by researchers at the USDA to understand how rising concentrations of CO<sub>2</sub> could impact cacao physiology (Baligar et al. 2005; Baligar et al. 2008). A climate-controlled greenhouse experiment was carried out to evaluate increased CO<sub>2</sub> (380 and 700  $\mu\text{mol mol}^{-1}$ ) concentrations and photosynthetic photon flux density (PPFD) on the growth, mineral nutrient uptake and nutrient use efficiency of cacao during its early growth stages (Baligar et al. 2005). The seedlings were grown in contrasting conditions for 57 days. The end results were overall very positive. Plants growing in the higher CO<sub>2</sub> concentration increased the uptake of all mineral nutrients, and tended to increase the shoot and root growth parameters measured (dry weight of roots, stem and leaves; stem height; leaf area; shoot/root ratio; leaf area ratio; and relative growth rate). In a follow-up study, independent short-term effects of PPFD, external CO<sub>2</sub> concentration and vapour pressure deficit (VPD) were investigated in three cacao genotypes (Baligar et al. 2008). The data showed that increasing CO<sub>2</sub> significantly enhanced photosynthesis when raised from 85 to 680  $\text{cm}^3 \text{m}^{-3}$ , yet minimally when increased from 680 to 850  $\text{cm}^3 \text{m}^{-3}$ . On the other hand, increasing CO<sub>2</sub> decreased stomatal conductance by about 65%, potentially decreasing transpiration. Thus, the authors concluded that increasing environmental CO<sub>2</sub> concentrations could probably improve cacao water-use efficiency.

The UoR in the UK has played a leading role in continuing to investigate how environmental changes in CO<sub>2</sub> concentration and soil water deficit, and the interaction between them, can impact cacao yield and the physiological determinants of yield. They carried out trials in climate-controlled greenhouse facilities run from a central computer system that could effectively simulate the predicted climatic conditions of cacao-producing areas. They evaluated traits such as photosynthesis, quantum efficiency, light saturation point, stomatal conductance, water-use efficiency and vegetative growth in response to long-term CO<sub>2</sub> elevation (700 ppm) and water deficit, with the aim of identifying genotypic variation in responses (Lahive 2015). Significant reductions in growth and photosynthesis, in response to water deficit stress, were observed, yet vegetative growth, maximum photosynthesis and water-use efficiency ( $A_{\text{max}}/g_s$ ) were significantly enhanced under elevated CO<sub>2</sub> in both juvenile Amelonado plants and mature clones (Lahive et al. in press; Lahive 2015). The magnitude of the response varied between young and mature plants; in mature cacao, elevated CO<sub>2</sub> helped to offset some of the negative effects of water deficit. Thus, there might be some responses associated with increased CO<sub>2</sub> that could potentially mitigate some of the impacts of drought in an environment with increased CO<sub>2</sub>. Both water use efficiency and quantum efficiency increased in response to elevated CO<sub>2</sub>.

Flowering, pod production and bean quality were also assessed in response to elevated CO<sub>2</sub> and water deficit in the different genotypes (Handley 2016). The objective was to assess the interaction of these climatic factors on cacao vegetative growth, photosynthetic parameters and yield components of different genotypes, by measuring the effects of environmental variables on flower and fruit development. Overall, reproductive responses resulting from water deficit and increased CO<sub>2</sub> were not as consistent as vegetative responses. Significant decrease in reproductive parameters to water deficit were observed in year one but not in the year two. Such inconsistencies were also observed for bean quality (fat content), pod growth rate, fresh pod weight and yield components.

In January 2017, as a follow-up to the research completed at the UoR, a new programme was initiated with a view to providing a more comprehensive understanding of climate change impacts on physiological responses, yield, and quality of cacao. The overall aim is to identify which physiological and morphological traits underlie the variation present in genotypic responses to climate change (specifically interactions between high temperature and CO<sub>2</sub>, and soil moisture deficit and CO<sub>2</sub>). Screening tools to aid in the identification of climate change resilient germplasm will also be developed as part of this programme. Physiological data from previous and future work will be used to build models that can predict changes in yield and plant performance in response to environmental variables. One component of the proposed evaluations is the establishment of multisite trials. These in-country trials would aim to evaluate the stability of traits identified in the greenhouse as important for conferring tolerance to water deficit or high-temperature stresses in field-grown cacao. These proposed evaluations will undoubtedly contribute valuable information towards understanding the many issues challenging cacao cultivation in the face of climate change, specifically by looking at how cacao genotypes respond in dissimilar environments.

There is also ongoing research at James Cook University in Australia, evaluating the possible interactions between increased CO<sub>2</sub> and increased temperatures in climate-controlled glasshouses. Future trials are expected to include interactions with water limitations (Lucas Cernusak, personal communication, 2017).

## 7 RECOMMENDATIONS

The information compiled in this report serves as a basis to understand the questions that remain regarding cacao responses to abiotic stresses and mechanisms, and the resources that are available to answer them. Compared to other important crops such as coffee, wheat, or maize, it is evident that the extent of work in relation to cacao physiology and responses to abiotic stresses is not reflective of its market value or importance to the livelihoods of millions of farmers. More research is still needed in order to achieve greater understanding of the complexities in cacao physiology and genetic diversity.

Although future climate predictions are worrisome, there is an expectation that enough genetic material is maintained within national and international collections that will allow for the establishment of improved resilient planting material. Some important guidance for next steps in research in this area includes the following:

- i. **Climate change models:** Conclusions from model projections, and the models themselves, need to incorporate knowledge on the diversity of cacao physiological responses and thresholds. The simulation models proposed by the UoR and WUR are in line with this concept. They aim to understand the impacts of climate change on plant functioning, yield and quality in cacao, and then synthesize the information into a **predictive physiological model** that understands the potential genetic diversity and variable response in cacao.
- ii. **Drought and temperature tolerance:**
  - a. There is a need to exploit confirmed genetic recombination and heritability potential towards the establishment of new and improved varieties in the new and ongoing work to evaluate the full potential of the heritability of select drought and temperature tolerance traits in cacao. Yet, more field validations are needed to increase the scope of understanding of resilience traits in cacao, and to generate information that can be easily conveyed to farmers.
  - b. A number of cacao cultivars have been identified as being drought tolerant, based on their capacity for **osmotic adjustment**. However, the literature also indicates that a cacao plant's capacity for osmotic adjustment might be limited in terms of providing comprehensive drought tolerance, and thus must be evaluated further, if designated as a basis for selection.
  - c. Research suggests that **stomatal sensitivity and conductance** might be an interesting target for future efforts engaged in breeding for drought and heat tolerance in cacao, especially in seedlings. Integrating high leaf-water status, rather than optimizing water use through water use efficiency, should be considered one of the priorities in breeding for more tolerant genotypes. Many of the ongoing trials are evaluating stomatal conductance, and as such will add important information to the discussion.
  - d. Observed **differential root growth**, specifically fine root growth, has shown potential as an attractive drought tolerance trait to investigate. However, given that a large proportion of the roots that branch out are located in the top 0.2 - 0.4 m soil, the probability of this trait serving as a method of escape by accessing deeper water reservoirs might be limited. It was noted that **companion planting** with deeper rooting species such as *Gliricidia* appeared beneficial

during times of water stress. This suggests an agroforestry-systems approach may also be useful in managing drought stress through increased ecosystem services. Researchers need to integrate past and ongoing work to support the notion that shade can enhance mitigation of drought and high temperature impacts, and generate better, more detailed shade management approaches.

- e. New trials are looking into the possibility of selected drought/heat tolerant **rootstocks** in conferring resistance to susceptible scions. There would be advantages to developing a collaborative approach to method selection and evaluation between the various institutes working in this area (INIAP, UESC, ICCRI, ICCRI, UoR, CRIG). Ongoing trials are also integrating other management practices for their potential to mitigate drought, like the amendments of mineral nutrition, specifically potassium. These studies, aside from focusing on how these amendments can mitigate drought impacts, should also evaluate the cost effectiveness, and viability of this option for farmers.
  - f. **Genetic responses.** Altering **polyamine levels** in cacao might result in enhanced tolerance to multiple stresses including drought. Many responses were shown to have been induced by environmental factors, even where inherent genetic induction was also responsible for the differential responses. Such results show promise in the potential to exploit such differences in breeding programmes.
- iii. **Genetic variation towards recombination:** Enough variation exists in the collections to explore, select and breed for superior more **photosynthetically efficient cacao canopies**. Breeding for this and other traits may improve the performance and sustainability of cacao under specific growing conditions. Studies have also looked at the mechanisms of trait inheritance, and numerous cacao plant attributes have been recorded. The combining potential of selected cacao cultivars, with high heritability and genetic variation in tolerance levels is promising, yet greater diversity needs to be included in future trials to get a sense of how broad heterozygosity and hybridization can really improve tolerance in cacao.
  - iv. Recent studies at the UoR and CU on cacao responses to **environmental changes and rising CO<sub>2</sub>** levels, carried out in climate-controlled glasshouses, are gathering novel data on interactions between expected water deficits and increased temperature, and rising CO<sub>2</sub>. Future multisite evaluation trials should aim to evaluate trait stability and responses under different climatic conditions whilst also identifying genotypic variation in the responses.
  - v. Similar types of investigations, especially on the evaluation of physiological traits, are ongoing. Another area that different institutions – the UoR, the CRC/UWI, and the Corporación Colombiana de Investigación Agropecuaria (CORPOICA) – mentioned they are currently engaged in, is in the development of screening methods. Institutes need to establish a network amongst themselves, and enhance inter-agency communication to avoid duplication and build upon the complimentary aspects and synergies of identified communalities.
  - vi. **A collaborative initiative towards climate resilience in cacao**, developed as a global public-private consortium, is needed to move forward in the face of coming challenges. The focus should be geared towards exploring and identifying promising genetic diversity in existing cacao collections and in farmers' fields, and to facilitate international access and benefit sharing of



resources to tackle the challenges faced by climate change without duplication of efforts. The consortium should bring together actors delivering improved planting materials to farmers: research partners, breeding networks, plant suppliers, the private sector, action networks and producers' associations.

- vii. It would be helpful to **standardize methodology, principles and selection criteria** to facilitate the comparison and sharing of results between institutions, and promote the development and exploitation of synergies between many organizations. It is through this exercise that we can more rapidly advance research and continue to build upon the understanding of the effects of drought and temperature, so that we can be better prepared to tackle the coming climatic challenges.
- viii. **Participatory approaches** are needed to incorporate local knowledge on resilient cultivars, and climate impacts. Looking forward, novel research methods that facilitate data gathering from farmers should be incorporated alongside traditional research methods, towards greater breeding efforts. Such innovations are already being used for other crops, and must be adopted in cacao cultivation to allow for the rapid generation of data and real-time evaluation of climate change impacts on production.

## 8 CONCLUSIONS

Despite all these research initiatives, when compared to other economically important crops, the extent of investigations and published material in relation to cacao physiology and responses to drought, temperature, or increased CO<sub>2</sub> stresses, there is limited information. The available literature does not reflect cacao's market value, or its importance to the livelihoods of millions of farmers. As was reflected in this report, there are copious studies on seedlings in greenhouses, yet a shortage of data to validate such findings in established field trials. This lack of field validation often results in a scarcity of meaningful information or enhanced genotypes for the farmers. It is imperative that future trials consider taking evaluations to field scale. Given that environmental pressures are only expected to increase with climate change, understanding the ways that cacao copes with increased CO<sub>2</sub>, drought and heat, is of major importance at the field level. Experiments need to be validated under natural field conditions in order to be representative of all the environmental interactions that can take place in cacao fields, aiming to include a wider genetic base. Field validations would increase the scope of understanding of the behavioural traits and adaptive capacity of cacao, and would provide information that can be easily conveyed to farmers.

Many of the frequently cited species distribution models in cacao are forecasting, based on the presence or absence of the crop, a pronounced loss in production suitability in cacao-producing areas, some to a greater extent than others, possibly through the combination of both increased temperatures and decreased water availability. Nonetheless, prediction models are only as good as their make-up and the quality of the data integrated into the model. For many regions, reliable climate data is unavailable, thus the modelling results obtained from such data should be considered with caution. In addition, many of the projections are not analysed in combination with the physiological adaptability or genetic diversity of cacao. These aspects must to be added into the discussion before definitive conclusions are made regarding the future of cacao production, otherwise misleading interpretations of models could be spawned leading to worrisome ramifications. New models and climate prediction exercises will be crucial to evaluate the impact of climate change on cacao production. Once the physiological adaptability and genetic variability are included into these proposed models, our discussions will be better directed to make decisions about the future of cacao breeding. These proposed models will be fundamental in guiding efforts towards the creation of more resilient cultivars.

While there is general agreement that cacao is currently affected by drought and higher temperatures resulting from climate change, the literature shows divergent results in terms of tolerance, response mechanisms, and the severity of environmental impacts. This could be a reflection of the small sample sizes, limited genotype comparisons, experimental conditions, or differing experimental methodology. Future trials should aim to increase not just the number of cultivars evaluated, but also comparisons between genetic groups. Regardless, the use of physiological parameters such as stomatal conductance, photosynthetic response, and osmotic adjustment seem to provide valuable information towards the level of adaptability in different genotypes, and are still the favourites in terms of candidate traits for evaluation in ongoing trials.

Management practices, such as increased shade, have been used by farmers for many years to mitigate heat. As demand for production has increased, many of these practices have been replaced

by more intensive production management practices. Nonetheless, studies have indicated a positive response from increased shade towards greater climatic resilience. The search for climate-resilient cacao should be interdisciplinary and multidimensional, and it should incorporate all aspects of production: adaptation strategies, improved management practices (shade, nutrition, soil health), and more resilient varieties created through international collaborations and climate-smart initiatives.

Overall, the bulk of the data suggests that the extent of how drought or heat affects cacao is determined by the individual genotype's response and inherent traits present that allow it to avoid, tolerate or escape the negative effects of water limitation. Thus, if the range of genetic diversity out there is fully exploited (*in situ and ex situ*), there is great potential for increased cacao resilience through selective breeding of tolerant genotypes. More field validation using a greater number of diverging genotypes is needed in order to fully understand the range of adaptability within the broad gamut of cacao genetic material, along with models that are not only based on presence and suitability, but also include information on the physiological thresholds and genetic diversity of cacao.

The key to resilience lies in tapping the genetic potential of cacao as a way to fully understand production limits and constraints. Exploration, understanding, and documentation of the potential of cacao's largely untapped genetic diversity is fundamental to keep the crop producing in perpetuity. It is only through a global collaborative effort that this will be achieved.

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## 10 APPENDICES

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	Cocoa Research Association Ltd./Cocoa Research (UK) Ltd. (CRA/CRUK)	Tony Lass
	ECOM Agroindustrial Corp. Ltd (ECOM)	Jason Green
	Independent (Ind.)	Rob Lockwood
	Mars, Incorporated (Mars - Global)	Isabella Van Damme
	Mars - Global	Martin Gilmour
	Mars - Global	Martin Hovorka
	Mondelēz International (Mondelēz)	Nick Cryer
	Mondelēz	Sara Boyd
	University of Reading (UoR)	Andrew Daymond
	UoR	Fiona Lahive
	UoR	Paul Hadley
<b>USA</b>	Mars - Global	Juan Carlos Motamayor
	Mars - Global	Keith Ingram
	Pennsylvania State University (PSU)	Mark Gultinan
	Purdue University (PU)	Robert Joly
	United State Department of Agriculture – Agricultural Research Service (USDA-ARS)	Bryan Bailey
	USDA-ARS	Dapeng Zhang
	USDA-ARS	Lyndel Meinhardt
	USDA-ARS	Osman Gutierrez
	USDA-ARS	Virupax Baligar
	United State Department of Agriculture - Foreign Agricultural Service (USDA-FAS)	Clemen Gehlhar
	United State Department of Agriculture- Tropical Agricultural Research Service (USDA-TARS)	Ricardo Goenaga
	WCF – Feed the Future Partnership for Climate Smart Cocoa	Ethan Budiansky
	WCF	Paul Macek
	WCF	Virginia Sopyla
<b>Venezuela</b>	Universidad de los Andes (UA)	Ramón Jaimez



## 10.2 Questions sent out to institutions listed in Appendix 10.1

These questions were initially sent out to the different institutions listed in Appendix 10.1 as a way of inquiring about past, current, and ongoing trials related to drought and temperature tolerance. Any information received from respondents related to current and ongoing trials is described in Appendix 10.3.

- Have you or your institution worked on cocoa drought or temperature tolerance investigations?
  - How so?
- Are you, or others in your institution currently carrying out investigations related to cocoa drought and temperature tolerance, or resilience to climate change?
  - If so, what is being evaluated?
  - What traits are being evaluated?
  - With what aim?
  - Are the trials being conducted at a laboratory, greenhouse/ nursery or field level?
- Has your institution published work on cocoa drought or temperature tolerance?
  - If so, can you list some of the most recent work?
- What resources are available to start and sustain a local/international field trial?
- Do the local laws prohibit the free exchange of plant material (import/export), do they allow the export of local plant material to other research stations?
- Would you, or someone from your institution be available for a follow up call?
  - If so, can you please provide your contact information?
    - Skype:
    - Email:
    - Cell #:

### 10.3 Institutions and research topics

Summary of information gathered from the different institutions, organized per country.

#### I. AFRICA

##### Ghana

<b>Institution:</b>	<b>Cocoa Research Institute of Ghana (CRIG)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Field b) Greenhouse
<p><b>Research summary:</b> CRIG has for many years been very active in conducting research on selecting and breeding for drought tolerance in cacao. There is ongoing work evaluating the effects of water deficits on assimilate partitioning and on various physiological characteristics (chlorophyll fluorescence, relative water content, leaf membrane stability, and leaf content of proline and phenol).            In other research, the institute is also looking at mineral nutrition as a management approach to alleviating drought through the addition of potassium. The data gathered so far, comparing ten varieties at seedling stage, is promising. Significant differences for all traits have been observed between the varieties tested, yet genotype differences suggest that not all varieties may benefit from the possible drought mitigation of applied potassium, or that different pathways are activated. A continuing field trial was due to be planted in June 2017.            In another study, a commercial super-absorbent polymer (SAP) is being tested under both screen-house and field conditions for its effect on enhancing soil moisture for young cacao survival. Specific rates that have a stimulating effect on plant growth were observed in pot studies. Field studies on the SAP were due to be concluded in June 2017.</p>	

##### Nigeria

<b>Institution:</b>	<b>Cocoa Research Institute of Nigeria (CRIN)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Germplasm collection b) Trained field officers c) Expert scientists (breeders, physiologists, pathologists, entomologists and agronomists) d) Collaborating institutions (University of Calabar, University of Ibadan and the International Institute of Tropical Agriculture)
<p><b>Research summary:</b> CRIN has carried out multiple trials in relation to cacao drought tolerance. Much of the ongoing work at CRIN is being conducted at greenhouse and laboratory scale. An ongoing collaborative project on screening for seedling establishment is being conducted by Dr Festus Olasupo (CRIN), Dr Osman Gutierrez (USDA-ARS, Miami), Dr Peter Aikpokpodion (University of Calabar) and Dr Sifau Adejumo (University of Ibadan). The core of the data gathered is focused on leaf sampling collected from water-stressed plants, to assay reactive oxygen species, free scavengers and amino acids that have been implicated in drought resistance in plants (proline and glutathione).</p>	

## Nigeria

<b>Institution:</b>	<b>International Institute of Tropical Agriculture (IITA)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<b>Research summary:</b> IITA is currently evaluating cacao genotypes for drought and heat-stress resistance in multiple field locations. A number of physiological traits related to drought resilience are being measured (stomatal conductance, root architecture, transpiration rate). In the near future, greater genomic tools, such as CRISPR technology will be implemented to improve stress resilience screening. IITA is also working in collaboration with WUR on several trials and projects (see WUR section for further details).	

## Nigeria

<b>Institution:</b>	<b>University of Ibadan (UI)</b>
<b>Research Status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<b>Research summary:</b> Research by Dr Abayomi Oyekale at UI has focused largely on the socio-economic impacts of climate change, farmers' adaptation mechanisms and risk insurance preferences. These studies were mostly carried out in Cameroon, Ghana and Nigeria, and are based on questionnaires. As an agricultural economist, the focus of his research has been on socio-economic impacts of climate change on cocoa farmers.	

## II. ASIA

### Australia

<b>Institution:</b>	<b>James Cook University (JCU)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Temperature-controlled glasshouses b) One hectare of land earmarked for an experimental cacao planting at Cairns Campus of James Cook University (JCU); the field trial is being led by Tobin Northfield. c) JCU also has a campus in Townsville and a research station in the Daintree region. Among the three sites, there is a considerable range in mean annual precipitation, from approximately 1,200 to 5,000 mm per year. These additional sites also have available land that could be planted with cacao to support an international field trial.
<b>Research summary:</b> James Cook University is investigating cacao responses to elevated temperatures and their	

potential interaction with elevated carbon dioxide (CO<sub>2</sub>). The objective of the current studies is aimed at evaluating whether an atmospheric rise in CO<sub>2</sub> can ameliorate some, or all, of the negative impacts of the expected higher temperatures. Researchers at JCU are looking at the plant physiological responses (vegetative growth, wet and dry biomass production, gas exchange, and secondary metabolites, among others) of cacao seedlings grown in high temperatures with and without elevated CO<sub>2</sub>. In the near future, they also would like to investigate the interactions with water deficit.

## India

<b>Institution:</b>	<b>Central Plantation Crops Research Institute (CPCRI)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Field b) Nursery c) Poly house d) Laboratory e) Cacao genebank
<p><b>Research summary:</b> Although at the moment they conduct limited trials on cacao climate resilience, the Indian Council of Agricultural Research/Central Plantation Crops Research Institute (ICAR-CPCRI) have been working on cacao drought tolerance for more than 20 years. In general, they are continuing to screen cacao varieties, looking for hybrids with high yield and drought tolerance. Recently, Dr Kacou from the Centre National de Recherche Agronomique (CNRA) of the Côte d'Ivoire worked on some trials related to cacao drought tolerance in collaboration with ICAR-CPCRI.</p> <p>Studies at ICAR-CPCRI are currently aimed at observing drought tolerance responses at the physiological, metabolic and enzymatic levels:</p> <ul style="list-style-type: none"> <li>• Physiological traits – stomatal resistance, conductance, transpiration rate, leaf water potential, photosynthesis, CO<sub>2</sub>, water use efficiency, chlorophyll fluorescence, epicuticular wax, among others.</li> <li>• Biochemical metabolites – total soluble sugar, amino acid, proline, protein etc.</li> <li>• Antioxidant enzymes – MDA, SOD, CAT, POX, PPO, etc.</li> </ul>	

## Indonesia

<b>Institution:</b>	<b>Indonesian Coffee and Cocoa Research Institute (ICCRI)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Greenhouse b) Field experimental station in 'low land and medium land' c) Cacao breeders d) Agronomist e) Soil scientist f) Technicians
<p><b>Research summary:</b> The Indonesian Coffee and Cocoa Research Institute (ICCRI) has been conducting research on mitigating drought conditions by developing technologies on soil conservation, shade management, and superior cultivar selections. Some previously selected clones are being evaluated under different field locations to confirm adaptability and performance. Traits used to assess drought tolerance are growth and yield performance.</p> <p>For many of the trials, selection of the hybrids was carried out in the greenhouse, establishment took place in the field for multi-location comparison, and laboratory work was carried out for the anatomical aspects. Some of</p>	

the outstanding clones identified so far have been KW 641, Sulawesi 3, and KEE 2; and hybrids ICCRI 08H (Sulawesi 1 x KEE 2) and (Sulawesi 3 x KEE 2).

In addition, the ICCRI are currently studying leaf anatomical characteristics that can be associated with drought tolerance. So far, stomatal traits seem to be the most promising anatomical characteristics identified. This leaf anatomy study is being carried out in collaboration with a master's degree student at Gadjah Mada University Yogyakarta, with a possibility of extending this trial in collaboration with the University of Reading.

An evaluation of the potential of rootstocks to confer drought tolerance is also currently being conducted. Researchers have already identified specific hybrids to be used as tolerant rootstocks.

### III. CENTRAL AMERICA AND THE CARIBBEAN

#### Costa Rica

<b>Institution:</b>	<b>Bioversity International</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Crop modellers
<b>Research summary:</b> Scientists at the Bioversity Costa Rica station, in association with ICRA, are working with species distribution modelling to assess the impacts of climate change on the suitability of 50 shade species widely used in cacao systems in Central America. The research assesses how changes in tree distribution (contraction or expansion) overlap with cacao areas vulnerable to climate change. The information generated provides insights into the potential of agroforestry adaptation plans for cacao cropping systems in Central America under future climatic conditions. It also provides information on which areas hold the most potential to develop or maintain diversified cacao agroforestry systems in the region.	

#### Costa Rica

<b>Institution:</b>	<b>Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Germplasm collection b) Fields c) Greenhouse
<b>Research summary:</b> While there are currently no specific trials being conducted to evaluate climate change resistance at CATIE, the centre has an immense amount of data, collected on a monthly basis over a period of 17 years (per tree), which can help identify physiological changes and link them to climatic variations. All this information is available in a very robust, trustworthy and available database for 42 clones. These data are available to be analysed to show correlations, and create models of responses based on years of data.	

### Costa Rica

<b>Institution:</b>	<b>Universidad Nacional de Costa Rica</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<b>Research summary:</b> Cacao climate resilience research at the Universidad Nacional de Costa Rica is aimed at identifying and helping to implement management practices that reduce the effects of climate change on cacao production. Researchers at the university are currently focusing their efforts in the Atlantic regions, working with producers that are members of the Centros de Procesamiento y Mercadeo de Alimentos (CEPROMA). This work is being carried out as part of a master's thesis in conjunction with Ghent University in Belgium and Pisa University in Italy.	

### Honduras

<b>Institution:</b>	<b>Fundación Hondureña de Investigación Agrícola (FHIA)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Collection of about 300 different cultivars b) Network with different producers c) Nurseries d) Laboratories
<b>Research summary:</b> FHIA is currently not conducting any trials, nor has it worked on topics related to cacao drought or temperature tolerance. Researchers have established some clones for further evaluation in areas considered very hot and arid for cacao, yet this was not designed as a trial, rather for observation. Their research has focused on the agronomic behaviour of cacao under agroforestry systems in areas considered appropriate for cultivation, genetic resistance to diseases, sexual compatibility and postharvest processes in cacao.	

### Trinidad and Tobago

<b>Institution</b>	<b>Cocoa Research Centre - University of the West Indies (CRC/UWI)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) The International Cocoa Genebank b) Equipment to measure agronomic, physiological and molecular traits c) Greenhouse and Shadehouse d) Fields, field plots with facilities for drip-irrigation e) Technicians f) Laboratories
<b>Research summary:</b> The CRC/UWI is a public research Institute associated with the University of the West Indies. In addition to the International Cocoa Genebank, CRC maintains several replicated field trials, demonstration farms and greenhouse trials. CRC has collected data over several decades related to yield, quality and disease resistance.	



Over the last three years, the CRC/UWI has been working on a modelling project entitled 'Climate Impacts and Resilience in Caribbean Agriculture: assessing the consequences of climate change on cacao and tomato production' (CIRCA), as part of the Climate Development Knowledge Network. This project has involved mapping areas where cacao is grown, and modelling the climate envelope needed for good production. This was combined with down-scaled climate data from the climate change prediction scenarios of the Intergovernmental Panel on Climate Change (IPCC), in order to assess the risks posed by climate change.

In addition to this modelling work, the CRC/UWI has collected data related to drought and temperature stress (stomatal conductance, leaf temperature, chlorophyll content, dark- and light-adapted chlorophyll fluorescence) in the field and in the greenhouse. Currently the CRC/UWI has one full-time PhD student, focused on measuring drought tolerance in mature trees at the International Cocoa Genebank (measuring traits related to water potential, chlorophyll fluorescence and leaf anatomy).

Using rhizotrons to profile quantitative root traits under optimum and water deficit conditions, researchers are also looking at root traits in greenhouse-grown seedlings. This work aims to compare accessions from different genetic groups and their responses to drought, by measuring parameters from mature trees in the wet and dry season. In the near future, they aim to combine all the gathered phenotypic data (above and below ground) with genotypic data in order to identify candidate genes for marker-assisted selection.

#### IV. EUROPE

##### *France/Peru*

<b>Institution:</b>	<b>Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<b>Research summary:</b> Currently, limited work is being conducted directly by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) on cacao drought or temperature tolerance. Dr Deheuvels is working on primarily aiding farmers and their production to be more resilient through enhanced management practices (cultivation, establishment, propagation), and on understanding how climate changes are affecting the cacao pollinator.	

##### *Germany*

<b>Institution:</b>	<b>University of Göttingen (UoG)</b>
<b>Area:</b>	<b>Europe</b>
<b>Country:</b>	Germany
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<b>Research summary:</b> Researchers at the University of Göttingen (UoG) conducted several studies on the ecology of cacao agroforestry systems in Indonesia (Sulawesi). Not all of them were explicitly related to drought tolerance, though this was one of the topics. However, they are currently not working on any projects related to cacao.	

### The Netherlands

<b>Institution:</b>	<b>Wageningen University and Research (WUR)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Crop modellers
<p><b>Research summary:</b> A variety of research is being conducted at Wageningen University and Research (WUR) related to cacao drought and temperature tolerance. They are currently building on the work of the Cocoa Fertilizer Initiative and are part of a consortium that was launched to establish a set of guidelines for integrated soil fertility management of cacao. The initiative is led by WUR and IITA/CGIAR, among others. Some of their soil nutrition work builds upon the hypothesis that mineral nutrition (in particularly potassium) can play a major role in drought tolerance in perennials.</p> <p>A large component of this initiative is on crop modelling. WUR has modelling expertise (and current models), as well as the knowledge on tropical tree ecophysiology that will be used to innovate and generate models that combine cacao physiology with the projected effects of climate change on production. This work builds upon an existing physiologically-based cocoa production model that can model potential and water-limited cacao production (Zuidema et al. 2005).</p>	

### United Kingdom (UK)

<b>Institution:</b>	<b>Cocoa Research Association Ltd. (CRA), Cocoa Research (UK) Ltd. (CRUK) and Ghana Cocoa Growing Research Association (GCGRA)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<p><b>Research summary:</b> The Cocoa Research Association Ltd. (CRA), with support from Mars, Mondelez and ICE Futures Europe, has been an active supporter of cacao genetic resources initiatives for many years. This industry association, and its predecessor, the Biscuit, Cake, Chocolate and Confectionary Association (BCCCA), has provided continuing support to the Cocoa Research Centre, University of the West Indies, Trinidad (CRC/UWI), and the maintenance of the International Cocoa Genebank, Trinidad (ICG-T), in collaboration with Prof P. Umaharan and the International Cocoa Quarantine Centre (ICQC) at the University of Reading (UoR).</p> <p>Cocoa Research (UK) Ltd. (CRUK) has just initiated a five-year (2017-2021) programme at the UoR that will allow for the continuation of their previous research on cacao and climate change. The previous project involved two PhD students who investigated the potential effects of climate change on the growth, physiology and bean quality of different genotypes of cacao. This research was conducted in state of the art controlled-environment glasshouse facilities at the UoR which allow the quantification of factors such as temperature, CO<sub>2</sub> and water stress. This continuing five-year project intends to make a substantial contribution to our understanding of the potential impact of climate change on cocoa production, and towards strategies to mitigate these effects. Information is further elaborated in the UoR section.</p> <p>CRUK has also funded postdoctoral work at the UoR to evaluate the physiological characteristics of accessions held in the ICQC for traits likely to affect yield potential and stress resilience. The project involved a study period at the ICG-T with the Cocoa Research Centre (CRC/UWI) to evaluate some of the same clones grown under field conditions.</p> <p>CRA managed a project supported by LNV Sustainable Cocoa Subsidy Scheme (Dutch Buffer Stock) and the Sustainable Tree Crops Program (STCP) from 2008 to 2011, looking at the importance of factors such as rootstock-variety effects and physiological performance of the variety during establishment and early growth,</p>	

especially under drought conditions.

In addition, the Ghana Cocoa-Growing Research Association (GCGRA) and CRUK have been supporting the breeding work of the Mabang Megakarya Selection Programme (MMSP), together with CRIG and partners. MMSP is a breeding programme based on field evaluation of large numbers of clones and crosses. One of the trials has been established as a 6-parent partial diallel, using as parents some of the clones that the UoR group identified as having contrasting water-use efficiency characteristics, with a view to looking at field evaluation and heritability.

## UK

<b>Institution:</b>	<b>University of Reading (UoR)</b>
<b>Resources available:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Temperature-controlled glasshouses b) Collection with over 400 genotypes c) International Cocoa Quarantine Centre (ICQC) d) Laboratory e) Physiologist
<p><b>Research summary:</b> Following on from a five-year project focused on elevated CO<sub>2</sub> and water deficit stress, Prof Hadley's team at the UoR is currently looking at genotype x environment interactions in cacao – specifically genotypic responses to water deficit, high temperature and elevated CO<sub>2</sub>, and the interactions between these factors under climate-controlled greenhouse conditions. Identification of specific traits conferring climate resilience is a strong focus of the work, as it will set a basis as to what traits breeding programmes should be targeting for resilience. With all the information generated, they are working towards the development of a physiological model to predict the impact of climate change on cacao yields and how selection for different traits may affect yields.</p> <p>Additionally, work was carried out by Dr Fiona Lahive to physiologically characterize and screen the ICQC germplasm collection for traits that are likely to affect cacao yield potential and stress resilience. Similar screening was carried out at the ICG-T with the CRC/UWI under field conditions. This work will soon be published by Prof Hadley's lab. The objective was to identify interesting materials for priority inclusion in breeding.</p>	

## V. NORTH AMERICA

### United States of America (USA) - Global

<b>Institution:</b>	<b>Mars/Asia</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	
<p><b>Research summary:</b> Mars is collaborating with several entities that have projects relating to cacao drought or temperature tolerance, such as the International Group for Genetic Improvement of Cocoa (INGENIC)/Asia-Pacific Cocoa Breeders Working Group, JCU, and the UoR. As part of the INGENIC/Asia-Pacific Cocoa Breeders Working Group, Mars is collaborating with breeders from the CPCRI in India to have access to drought-resistant clones.</p> <p>Additionally, Mars is collaborating with JCU in Cairns, supporting research conducted by a PhD student, which focuses on the interaction between increased CO<sub>2</sub> and hotter temperatures on cacao, as well as coffee and cassava.</p> <p>Mars is also in the process of installing lysimeters in Ecuador that will allow researchers to measure transpiration rates of cacao and responses to nutrients. Initial research will be conducted with clone CCN51, but</p>	

other clones might be studied in the future.

Mars has supported two PhD research theses at the UoR, and is involved in the management of the new five-year programme through CRUK; these studies are covered under the work of the UoR. It is also a co-founder of MMSP – a breeding programme in Ghana that has a water-stress trial – and part of the World Cocoa Foundation's (WCF's) Climate Smart Cocoa Programme.

## USA

<b>Institution:</b>	<b>United States Department of Agriculture (USDA): Beltsville, Puerto Rico</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Cacao collection b) Breeders c) Agronomists d) Laboratories
<p><b>Research summary:</b> With the collaboration of both national and international players, the United States Department of Agriculture (USDA) is currently awaiting approval for the funding of a five-year programme. The new project merges two previous projects, one of which involved an assessment of genetic diversity and crop management, with the evaluation of cacao germplasm for drought tolerance and other abiotic stresses. One of the overall objectives is to characterize and evaluate cacao germplasm for tolerance to soil water deficits in order to identify promising clones for breeding efforts. They plan to evaluate cacao at the physiological level (gas exchange among others) and molecular level using specific genomic approaches like SNP markers and RNA sequencing to identify candidate genes. They hope to develop SNP markers to assist with germplasm evaluation and selection.</p> <p>One of the sites where screening and evaluation will potentially take place is at the Tropical Agricultural Research Service (TARS) station of the USDA in Mayaguez Puerto Rico, where the intention is to screen cacao genotypes from the USDA collection for drought and temperature tolerance under field conditions in the semi-arid zone of Puerto Rico.</p>	

## VI. SOUTH AMERICA

### Brazil

<b>Institution:</b>	<b>Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura Cacaueira (CEPEC/CEPLAC)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Cacao collection b) Fields c) Greenhouse
<p><b>Research summary:</b> At the Centro de Pesquisas do Cacau/Comissão Executiva do Plano da Lavoura Cacaueira (CEPEC/CEPLAC), they have worked and are working on several drought tolerance trials. Much of the work on drought is being carried out in collaboration with the Universidade Estadual de Santa Cruz (UESC), in Bahia Brazil. Projects range over the different aspects of cacao breeding and selection for drought tolerance, but with the focus on understanding the molecular, physiological and biochemical basis of resistance. Two master's degree students are currently carrying out trials in relation to screening and heritability of several physiological tolerance traits. Thus far, SPA-5, SIAL-70 and TSH-516 have been identified as resistant.</p> <p>At present, there are ongoing evaluations to understand the genetic variability and heritability of cacao</p>	

drought tolerance using the same progenies in the field and in the greenhouse. The goal is to estimate genetic variance and heritability associated with specific traits, and to identify some possible correlations (field vs. greenhouse, drought vs. yield components, resistance, etc).

Given the severe drought that has occurred recently in this production region, efforts have been made to assess clones in farmers' fields that have endured such extreme conditions in the hope of identifying new tolerant materials. Selections are underway: 80 clones have already been selected and are being cloned for further evaluation.

### Brazil

<b>Institution:</b>	<b>Universidade Estadual de Santa Cruz (UESC)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Greenhouses b) Laboratory equipment c) Master's and PhD students
<p><b>Research summary:</b> For the last five years, researchers at the Universidade Estadual de Santa Cruz (UESC) have been working towards identifying cacao cultivars that can better tolerate the increasingly longer periods of water deficit. The team comprises José Olímpio (soil scientist), Alex Alan Furtado de Almeida (Physiologist) and Dario Ahnert (geneticist). The research has focused on the genetic, physiological and molecular aspects of cacao drought response, with evaluations mostly conducted at greenhouse level. They aim to establish a protocol for selection under greenhouse conditions (selection screening before taking the cultivars to the field). In collaboration with CEPLAC, the team has already published work on the evaluation of 36 progenies from clones of different geographical origins exposed to water deficit. Some of the traits that they evaluated include hydraulic conductivity, chemical signalization, gas exchange, soil and leaf water potential, gene expression, proteome, electrolyte leakage, lipid peroxidation, oxidative stress, chemical composition (starch content, total soluble sugars and reducers in leaves and roots), osmoregulation (leaves and roots), and root architecture, etc. In the near future, mRNA-Seq libraries and computational biology will also be included in evaluations.</p> <p>New studies are evolving to evaluate the scion-rootstock interaction with respect to drought tolerance.</p>	

### Colombia/Nicaragua

<b>Institution:</b>	<b>Centro Internacional de Agricultura Tropical (CIAT)</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Modellers
<p><b>Research summary:</b> At the Centro Internacional de Agricultura Tropical (CIAT), researchers focus mainly on using species distribution models to model the effects of climatic variability in the suitability of cacao production. Using this approach, they have published several articles discussing what areas are prone to becoming unsuitable for future production in areas of West Africa and in the Asia Pacific region. Ongoing work is moving forward to apply these modelling procedures to evaluate impacts on production in Central America and Peru. CIAT's modelling group is working closely with the WCF and their Climate Smart Programme.</p>	

## Colombia

<b>Institution:</b>	<b>Colombian Corporation for Agricultural Research (CORPOICA)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Laboratories b) Palmira research stations with around 500 hectares and nurseries c) Research stations with contrasting environmental conditions (dry or wet conditions) d) Fields e) Nurseries f) Researchers
<p><b>Research summary:</b> There is new interest at the Colombian Corporation for Agricultural Research (CORPOICA) to study cacao drought or temperature tolerance. They are still in the initial stages of trial development. As an initial step, they want to choose representative accessions and create a standardized screening platform to identify contrasting responses. The plant breeding programme is located at two research stations (La Suiza-Santander and Palmira-Valle del Cauca).</p>	

## Ecuador

<b>Institution:</b>	<b>Instituto Nacional de Investigaciones Agropecuarias (INIAP)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Phichilingue stations comprise a very genotypically diverse cacao collection
<p><b>Research summary:</b> One of the projects at the Instituto Nacional de Investigaciones Agropecuarias (INIAP) aims to elucidate the physiological effects of a progressive drought over a six-month period. As such, they are collecting data on relative water content, CO<sub>2</sub> assimilation rates, stomatal conductance, instantaneous water use efficiency, relative quantum yield of PSII, non-photochemical quenching coefficient, electron transport rate and leaf nutrient content (nitrogen, phosphorous, calcium and magnesium). They expect to be able to select clones tolerant to water deficit by identifying differentiated physiological responses and yield.</p> <p>In a different continuing trial, rootstocks are compared to assess the physiological and biochemical aspects of the rootstock–scion interaction under water deficit. The objective is to better understand the effect of the rootstock’s physiology on the main physiological processes of the cacao scion when under water deficit. This evaluation was carried out in the greenhouse but is now being conducted in a field trial.</p>	



## Peru

<b>Institution:</b>	<b>Instituto de Cultivos Tropicales (ICT)</b>
<b>Research status:</b>	<input type="checkbox"/> Past work on cacao drought or temperature tolerance <input type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Greenhouse b) Fields
<b>Research summary:</b> At the Instituto de Cultivos Tropicales (ICT) in Peru, trials are currently underway to assess drought tolerance in cacao by looking at how drought affects the growth and development of the plant (roots, stem and leaves), and how it impacts stomatal conductance. Additionally, they are evaluating the possible contribution of an endophytic fungus in the induction of drought resistance responses. The trials are being conducted at both greenhouse and field levels.	

## Venezuela

<b>Institution:</b>	<b>Universidad de los Andes</b>
<b>Research status:</b>	<input checked="" type="checkbox"/> Past work on cacao drought or temperature tolerance <input checked="" type="checkbox"/> Ongoing work on cacao drought or temperature tolerance <input type="checkbox"/> Proposed work to be initiated on cacao drought or temperature tolerance
<b>Resources available:</b>	a) Greenhouse b) Fields
<b>Research summary:</b> Several studies were published evaluating numerous Criollo-type Venezuelan cultivars under an agroforestry system. The main objective of those studies was to evaluate the ecophysiological traits (such as photosynthetic capacity) of different Criollo cacao cultivars during rainy and dry seasons in the southern region of Maracaibo Lake Basin. A continuation trial is ongoing to assess long-term effects.	

## 10.4 Glossary of terms

Term	Definition
CRISPR	'CRISPR' (pronounced 'crisper') stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are the hallmark of a bacterial defense system, forming the basis for the popular CRISPR-Cas9 genome editing technology. In the field of genome engineering, the term 'CRISPR' is often used loosely to refer to the entire CRISPR-Cas9 system, which can be programmed to target specific stretches of genetic code and to edit DNA at precise locations. These tools allow researchers to permanently modify genes in living cells and organisms and, in the future, may make it possible to correct mutations at precise locations in the human genome, to treat genetic causes of disease. (Broad Institute of the Massachusetts Institute of Technology (MIT) and Harvard).
Diallel cross	A Diallel-type cross consists of all possible crosses between a number of varieties. Reciprocal crosses, and the selfed parents, may or may not be omitted. In a full diallel, all parents are crossed to make hybrids in all possible combinations (Gilbert 1958).
Drought	A decrease in the water inputs (precipitation, irrigation) into an agro/ecosystem over time that is sufficient to result in plant soil water deficit (Gilbert and Medina 2016).
Drought avoidance/escape	A plant may avoid soil water deficit despite a lack of water inputs. Examples include plants that explore deeper soils, or have slow root growth leaving water for later in the season, as well as plants that conserve water through lower leaf area or transpiration, or match phenology to the wet season (Gilbert and Medina 2016).
Drought stress avoidance	Plants that avoid soil water deficit may have distinct physiological mechanisms that encounter soil water deficit but avoid physiological stress through osmotic adjustment, water storage in organs, or root isolation from soil (Gilbert and Medina 2016).
Factorial cross	In a factorial type cross, each member of a group of males is mated to each member of a group of females.
Osmotic adjustment	Osmotic adjustment is defined as the active accumulation of solutes or osmolytes to allow greater solute potentials in cells (Gilbert and Medina 2016).
Quantum efficiency	Unit of CO <sub>2</sub> fixed per unit of light absorbed.
Rhizotrons	Rhizotrons are transparent wall techniques that allow researchers to observe the roots while they are growing.
Water-use efficiency	Water-use efficiency gives a measure of CO <sub>2</sub> uptake per unit of water lost, or biomass accumulated per unit of water used.



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