THE BELGIAN DEVELOPMENT COOPERATION .be

Bioversity International

KU LEUVEN

More fruit for food security: developing climate-smart bananas for the African Great Lakes region.

Technical report (July 2019-November 2020)

Field trial in Mbarara, Uganda, mulched with swamp grass to reduce weeds and soil moisture loss. Photo credit: Bioversity International/L. Machida

Executive summary

Banana (*Musa* spp.) is an extremely important crop in the Least Developed Countries, providing a staple food for more than 400 million poor people. Environmental constraints (especially drought) globally cause estimated yield losses of 37-61 million tonnes annually, worth at least US$13-22 billion, with a third of these losses incurred in Africa.

The overarching goal of the project is to identify climate-smart banana cultivars that are the most suited for the specific agro-eco zone to help close the environmental yield gap in different areas. This project focuses on the African Great Lakes region, which is one of the poorest areas in the world, encompassing Burundi, north-eastern Democratic Republic of Congo (DR Congo), western Kenya, Rwanda, north-western Tanzania, and Uganda. The region is also one of the most important banana-growing areas in the world and is home to a unique group of banana cultivars the East African Highland Bananas (EAHB).

To support this research, we rely on Bioversity International's Musa Germplasm Transit Centre (ITC), hosted at the Katholieke Universiteit Leuven (KU Leuven) in Leuven that houses germplasm samples representing more than 1600 banana cultivars and 29 *Musa* wild relatives. Together they provide a substantial potential gene pool to find the existing germplasm that is better adapted to the current and future agro-environment. In Work Package (WP) 1, we have characterised the different agro-eco environments in the Great Lakes Regions, investigated the local socio-cultural preferences of male and female farmers, and determined the impact of suboptimal temperatures on the growth and development of 104 different gene bank accessions by high throughput screening in our BananaTainer. In WP2, we have investigated the impact of water deficit on a selected set of cultivars in our greenhouse phenotyping platform Phenospex and within the framework of the EU project EPPN 2020 on the European phenotyping platform Phenodyn. In WP3, we have investigated environmental impact on the yield of 3 selected cultivars in field trials located on 5 different agro-eco zones and have zoomed in on 3 crop cycles in collaboration with the National Agricultural Research Institute of Uganda (NARO). In WP4, we have characterised the chromosome structures of an important drought tolerant group, sequenced the genome of one reference ABB cultivar Cachaco, and we quantified the transcriptome and proteome of the stomata, a crucial cell structure in the leaves determining the water usage and growth. In WP5, we have shared and disseminated our knowledge by making our data available on our web platform MGIS and GIGWA and on PRIDE, by explaining our research on national television, radio and blogs, by publishing our scientific papers in peer reviewed open access journals and, by training Bachelor, Master's, and PhD students.

As the work was organised in five complementary Work Packages (WPs) we will also report our findings in this format:

● WP1: Analysis of the multiplicity in the region in terms of environmental constraints and socio-economical needs and high throughput assessment of the growth potential of relevant ITC gene bank accessions

In this project we have developed high-throughput screening methods, and deployed these to assess the growth potential of numerous banana cultivars for the current and future growing conditions in the Great Lakes region. The East African Highland Banana (EAHB) is said to be endemic to the region with no clear equivalent elsewhere in the world (Karamura et al., 1998). A key factor responsible for the group's distribution is the altitude. The plant grows comfortably within 1000-2000 masl. Those altitudes form an agro-ecological zone with relatively low temperatures and have thus a lower evaporative demand, meaning that due to the lower temperatures, less water is needed to sustain the growth. East African Highland Bananas have a low water use efficiency (van Wesemael et al., 2019). In other words they are big water consumers. Below 1000 masl, as the temperatures raise, the plants are more stimulated to grow and start consuming more water. As a result the water in the soil is lost rapidly leading to water deficit. So due to their water spending nature, East African Highland Bananas can not thrive outside the highlands. Even in highland areas with a longer dry season they seem to disappear from farmers' fields (Figure 1, Figure 2, Table 1). While the highlands might give protection from losing too much water, growth and development is also slowed down because of the lower temperature. The average optimal annual mean daily temperature for banana has been modelled to be 26.2°C (Machovina and Feeley, 2013). Areas having three or more months with an average daily temperature below 13 °C are not suitable to grow bananas (Calberto et al., 2015). Above 2000 masl, the temperature becomes indeed too low so that the plants are not stimulated enough to grow. Depending on the altitude, we calculated the current average daily temperature of the banana growing regions to vary between 16 and 25 °C (Figure 1) (van Wesemael, 2019). Our hypothesis is that there is diversity within the different bananas in terms of growth retardation due to suboptimal temperatures and in terms of water use efficiency and drought tolerance. Hence there is an imperative need to understand the cultivar specific plant responses towards temperature and towards water deficit so that we can recommend the cultivar suitable for the particular agro-eco zone now and in the future! The intensity of cultivation and the specific cultivar profiles are not only steered by the agro-ecological conditions (Table 1), but the local culture and other socio-economic forces ae also found to play a role (Marimo et al., 2020). So understanding user preferred traits is important for the successful introduction and adoption of alternative and new cultivars banana cultivars. We analused different banana products, the cultivars used to make those products and the preferred characteristics as reported by men and women farmers in different regions of Uganda and Tanzania. Qualitative data from 23 focus group discussions (FGDs) conducted in six districts (Mbarara and Luwero in Uganda; and Bukoba, Meru, Moshi and Rungwe in Tanzania) were coded and analysed (Marimo et al., 2019).

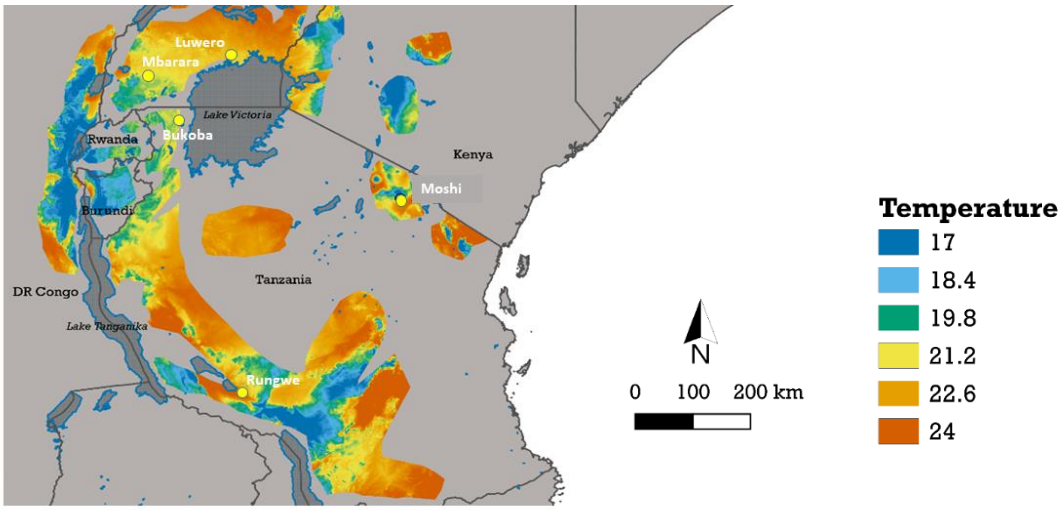

Figure 1: Average day temperature in the banana growing areas of the Great Lakes region during the wet season March-April**.** Five different areas have been studied in detail for on farm cultivar diversity and on station trials and are highlighted: Luwero, Mbarara, Bukoba, Rungwe and Moshi.

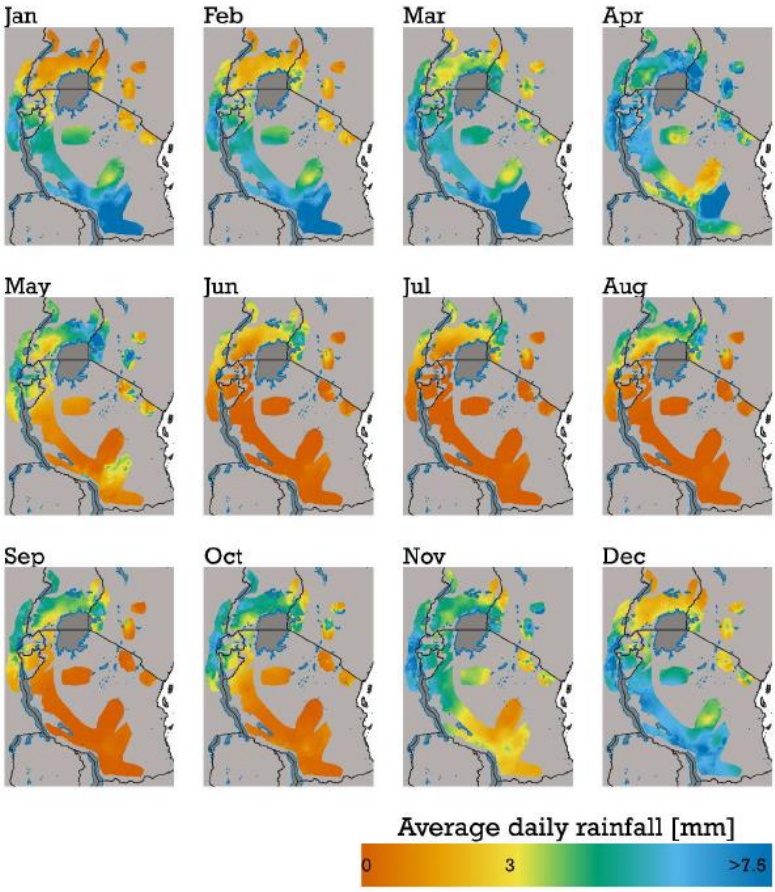

Figure 2: Average daily rainfall per month.

Table 1: Analysis of local usage per agro-eco zone

cultivar group	Genome constitition	# ITC accessions tested in the BananaTainer	ratio of local diversity usage*					
			Uganda		Tanzania			
			Luwero	Mbarara	Bukoba	Meru	Moshi	Rungwe
EAHB	AAA	34	0,76	0,89	0,55	0,32	0,10	
Mchare	AA	7			0,01	0,04	0,25	
Plantain	AAB	4	0,03		0,04	0,08	0,02	0,34
Cavendish	AAA	4			0,02	0,17	0,08	0,02
PisangAwak	ABB	4	0,01		0,06	0,03	0,02	0,20
Pome	AAB	3			0,01	0,08	0,07	0,07
Kamaramasenge	AAB	1	0,05	0,04	0,07	0,06	0,07	
Red	AAA	3		0,02				0,09
Ibota	AAA	2	0,03	0,02	0,08			
Bluggoe	ABB	4	0,02	0,01	0,03	0,04	0,03	0,07
GrosMichel	AAA	1	0,01	0,01	0,04	0,03		

* The ratio was determined by visiting farms and interviewing male and female farmers. A list with all the cultivars names was made per region and the ratio displays how many of the locally used cultivars belong to which group.

Using the BananaTainer, the ITC Germplasm collection (Bioversity International) has been efficiently used to screen the ITC gene bank for suitable plant material to meet current and future challenges. 104 ITC accessions have been analysed to determine their optimal temperature zone (Appendix 1). The set of 104 accessions represents 5544 individual plants that have been so far evaluated under different simulated agro-eco zones. This set of plants is a strategic selection from the ITC gene bank that has been multiplied based on the following criteria: (i) endemic to the region and thus socio-economically accepted, (ii) improved hybrid from a breeding program, (iii) important diploid parent for breeding and/or (iv) water efficient and drought tolerant accession (Appendix 1, Table 1). The impact of different temperatures in day and night periods on the growth responses of different organs has been investigated. Our results corroborated the hypothesis of diversity within the banana cultivars. The average optimal mean daily temperature across all tested cultivars was 26.7°C with an optimal cultivar specific growing temperature ranging between 20 and 30 °C (Figure 3).

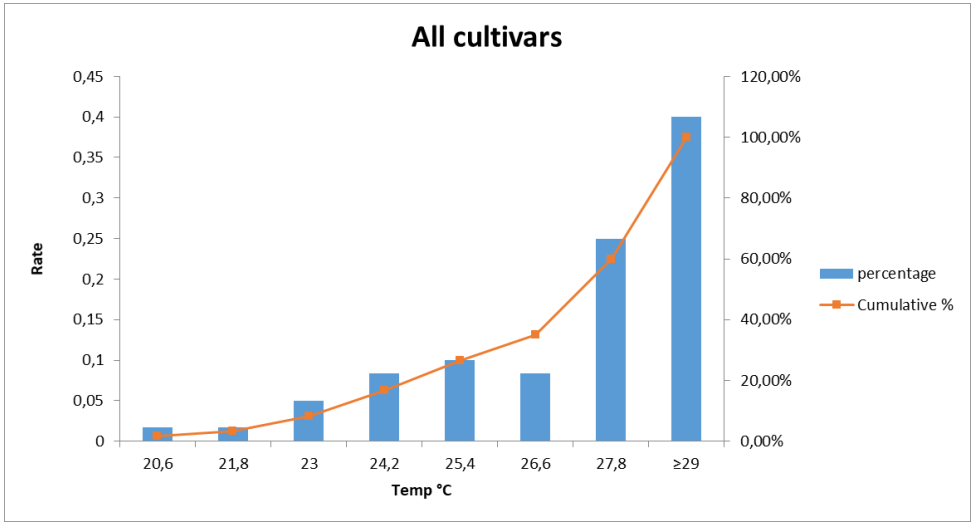

Figure 3: Temperature at which the specific cultivars show the highest growth. The average optimal mean daily temperature across all tested cultivars was 26.7°C with an optimal cultivar specific growing temperature ranging from 20 to 30 °C

● WP2: Phenomes underlying differences in growth.

Ideally we are looking for cultivars displaying good growth behaviour at the lower temperature ranges common for the great lakes region, that do not suffer too much from the (bimodal) drought periods also typical for the region, and possess a pleasant taste and quality to meet the

needs for the region. Taking into account the current and future climate change, we aim to look to identify and understand drought tolerant cultivars and have been investigating water saving mechanisms that are most likely genetically inherited.

Reactions towards light

Plants have openings in their leaves called 'stomata' that they can open and close. When plants receive light, they are genetically programmed to open their stomata to lose water and to grow. We have shown that there is variation in daily transpiration and growth rate and that the growth rate is perfectly correlated to the daily transpiration (Eyland et al in preparation). When there is a risk of losing too much water, the light signal can be overruled and water is saved. Our hypothesis was that EAHB have a tendency to "take risks": they are very enthusiastic to react to light and moreover, they wait to overrule the light signal when water becomes limiting. Indeed we have shown that EAHB plants respond faster and in a greater extend to light than the drought tolerant ABB varieties (Eyland et al, in revision, Figure 4).

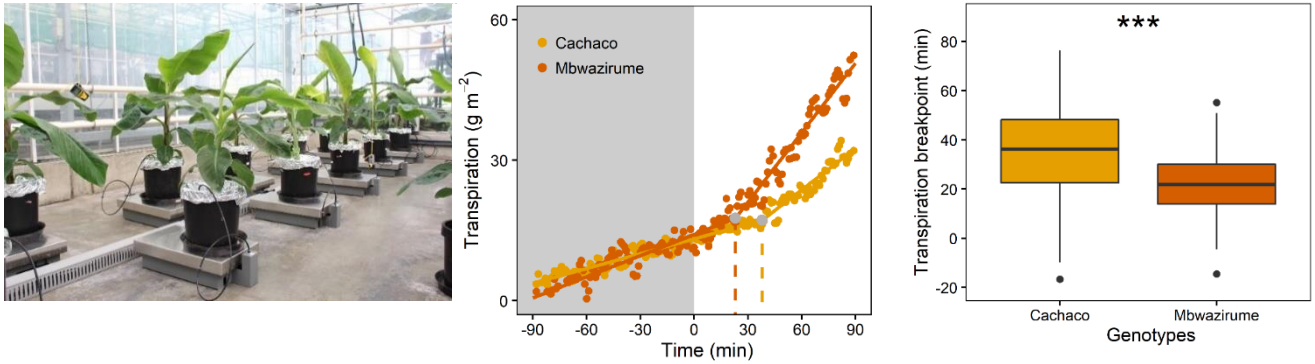

Figure 4: Light driven stomatal responsiveness. When switched to higher light intensities (e.g. at dawn or when a cloud is disappearing) stomata increase their conductance and thereby increase the capacity to grow. However, this opening is also associated with a greater water loss. When we induce a switch to higher light intensities, we see a clear difference between opportunistic behaviour in the EAHB cultivar Mbwazirume. The EAHB cultivar opens the stomata very fast (in around 20 min) resulting in a high transpiration rate while the conservative ABB cultivar Cachaco opens the stomata much slower (in around 35 min) and has a lower water loss. Water loss is monitored in our greenhouse where every plant is placed on a precise balance.

The reason why plants can grow when they receive light is because they can do photosynthesis. Photosynthesis is the process where light energy is used transform atmospheric CO_2 and water into organic plant material, our food. The light intensity defines in a way how fast the plant can grow and how much water is needed. We have modelled the extent of photosynthesis a banana plant can perform when receiving a certain amount of light (Figure 5). We discovered that the stomata of bananas overrule the light signal in the afternoon, resulting in a higher water use efficiency (van Wesemael et al., 2019). We investigated eight genotypes that had the best growth potential under water limiting conditions. Of

the eight investigated cultivars, five had an ABB genome constitution. The ABB genotypes had a lower maximal transpiration rate, kept this maximal transpiration for a shorter time and consumed less water per day (van Wesemael et al., 2019). The limitation of photosynthesis by the stomata in the afternoon especially contributed to the overall water use efficiency (Eyland et al, under revision, Figure 5). The stomata of the ABB cultivar Cachaco are limiting the photosynthesis and are anticipating excessive water loss. This is the kind of behaviour that is needed to be able to survive in the warmer and drier lowlands or the future warming of the highlands.

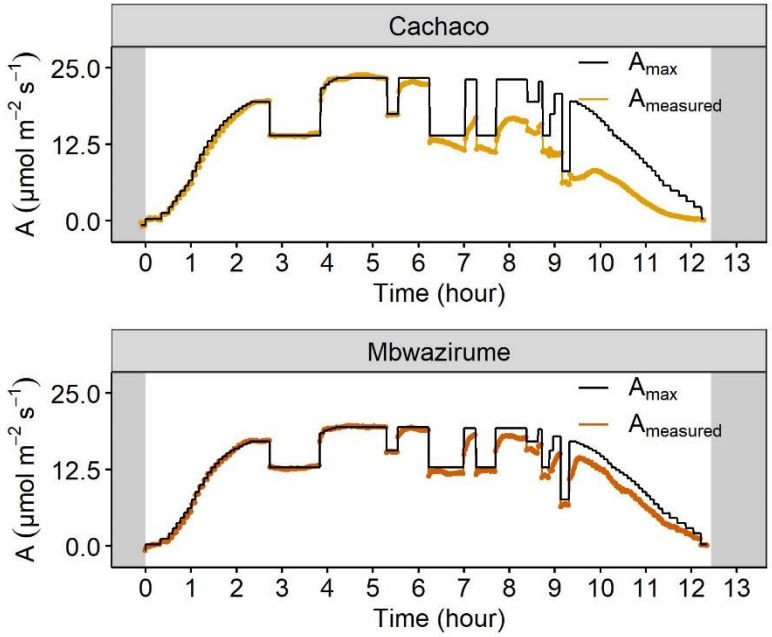

Figure 5: Light driven potential to perform photosynthesis (A). Plants have been exposed to different light intensities during the day and the maximal photosynthesis capacity corresponding to this light intensity has been modelled (A_{max}). In the afternoon (after 6 hours of light) the stomata do not respond as enthusiastically to the same light intensity, and the measured photosynthesis is lower. The stomata of the ABB cultivar Cachaco are limiting the photosynthesis to avoid excessive water loss. This is the kind of behaviour that is needed to be able to survive in the warmer and drier lowlands or in the highlands that have a long dry season.

Figure 6 shows the actual response of both cultivars to a diurnal changing light intensity. It is obvious that an EAHB consumes much more water when exposed to identical environmental conditions.

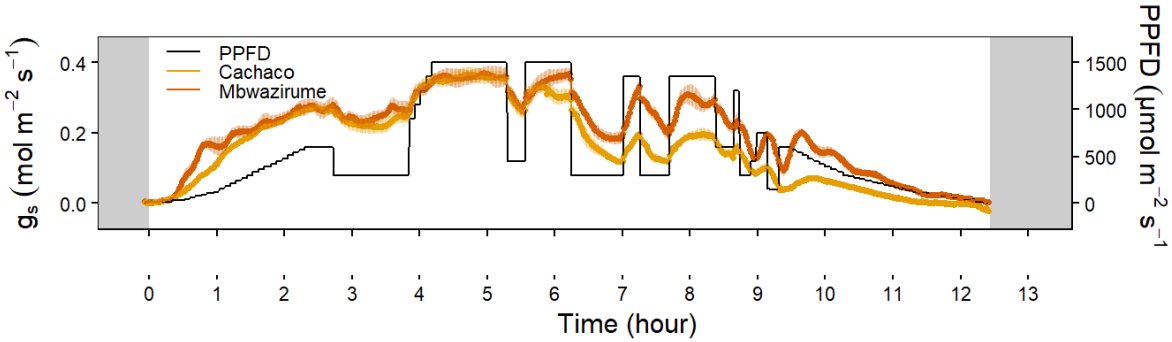

Figure 6: Stomatal water loss (g_s) in function of the light intensity (PPFD) and time of the day. Not only at whole plant level but also at the stomatal level Mbwazirume is faster to respond to light and loses more water at dawn. Moreover, Mbwazirume significantly loses more water in the afternoon.

Reactions towards dryer soil

Progressive soil drying makes it for the plant more difficult to extract water from the soil. At a certain soil level and thus resistance, the plant roots produce a hormone that is transported to the leaves making the stomata close and preventing the plant from drying out. Cachaco has more strict, conservative, control over the transpiration under progressive soil drying, closing stomata at higher soil water contents compared to Mbwazirume (Figure 7). Assuming both cultivars have the same potential to extract water from a stored water volume, Mbwazirume is predicted to reach its full growth limiting soil potential earlier and so Cachaco will be able to continue to grow longer during the dry season. Saving water for later is a reasonable drought avoidance strategy.

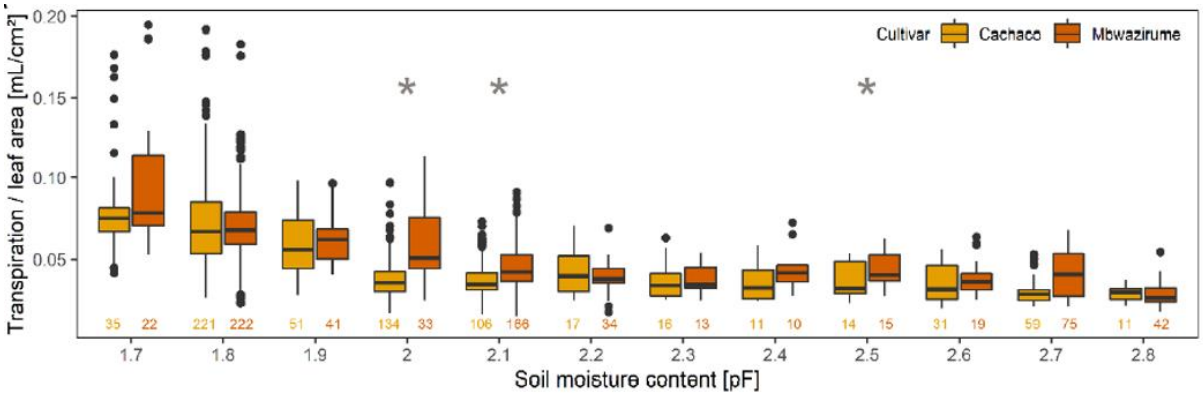

Figure 7: Water loss per area plant in relation to the soil water content. Cachaco has more strict, conservative, control over the transpiration under progressive soil drying, closing stomata at higher soil water contents compared to Mbwazirume. The biggest difference is observed at a soil water content of pF 2. The higher the pF Value, the lower the soil water content.

In the framework of the European project EPPN, we gained access to the high throughput phenotyping platform Phenodyn managed by INRAE Montpellier (Berthezene et al., 2015). This platform has with 400 balances more than 16 times the capacity of our Phenospex platform. In the framework of this project and the Global Diversity Trust prebreeding project, we phenotyped 4 different banana cultivars (AAA and ABB) and 6 different diploid M. acuminata (A) and M. balbisiana (B) bananas. In total, 15 gene bank accessions have been phenotyped. Continuous weight measurements combined with high-throughput imaging resulted in excellent growth prediction and detailed time courses of transpiration, derived transpiration responses to light, VPD and soil water potential (Eyland et al., 2020) (Eyland et al , in preparation).

● WP3: Field validation.

Crop variety evaluation is critical in decision-making in crop variety release, crop seed marketing or distribution and generating crop variety recommendations for farmers (Brown et al., 2020). So far during the current project, the on-farm diversity of 5 different agro-eco zones have been studied across Uganda and Tanzania (Marimo et al., 2020; Marimo et al., 2019). Three representatives with an optimal BananaTainer calculated mean temperature of 23,7°C, 28,4 °C and 29°C (respectively the dessert banana Williams, the EAHB (Mbwazirume) and the ABB Bluggoe (Cachaco)) have been studied in the field in 5 different locations (Figure 1). In Tanzania: Moshi has an annual mean temperature of 19.6°C, and an average total precipitation of 1546 mm per year that is spread in a bimodal season (Jan-Feb and Aug-Oct). Rungwe has an annual mean temperature of 20.7°C, and an average total precipitation of only 1050 mm per year, but one long dry season (May-October)! Bukoba has an annual mean temperature of 21.2°C, and an average total precipitation of only 917 mm per year with one dry season (June-September).

In Uganda: Luwero has an annual mean temperature of 21.5°C, and an average total precipitation of 1064 mm per year that is spread in a bimodal dry season (Jan-Feb and Jul-Aug). Mbarara has an annual mean temperature of 20.9°C, and an average total precipitation of 1132 mm per year that is spread in a bimodal dry season (Jan-Feb and Jun-Aug).

The rate of development of a banana plant is influenced by temperature (Turner, 1990). Since in the highlands environment temperature is low but stable, the short term growth and yield are predicted to be more a function of water supply. However, in contrast to water, the temperature cannot be controlled by farm management. It is clear that the African highland zone is suboptimal (Figure 1) also for the 3 investigated varieties. A lower temperature prolongs the crop cycle. The sides with the highest annual mean temperature, Luwero and Bukoba have the shortest crop cycle (Figure 8). Cachaco belongs to the ABB subgroup. As mentioned in WP 2, that group is predicted to require a lower maximal transpiration and water consumption per day but also produces a less vigorous yield (van Wesemael et al., 2019).

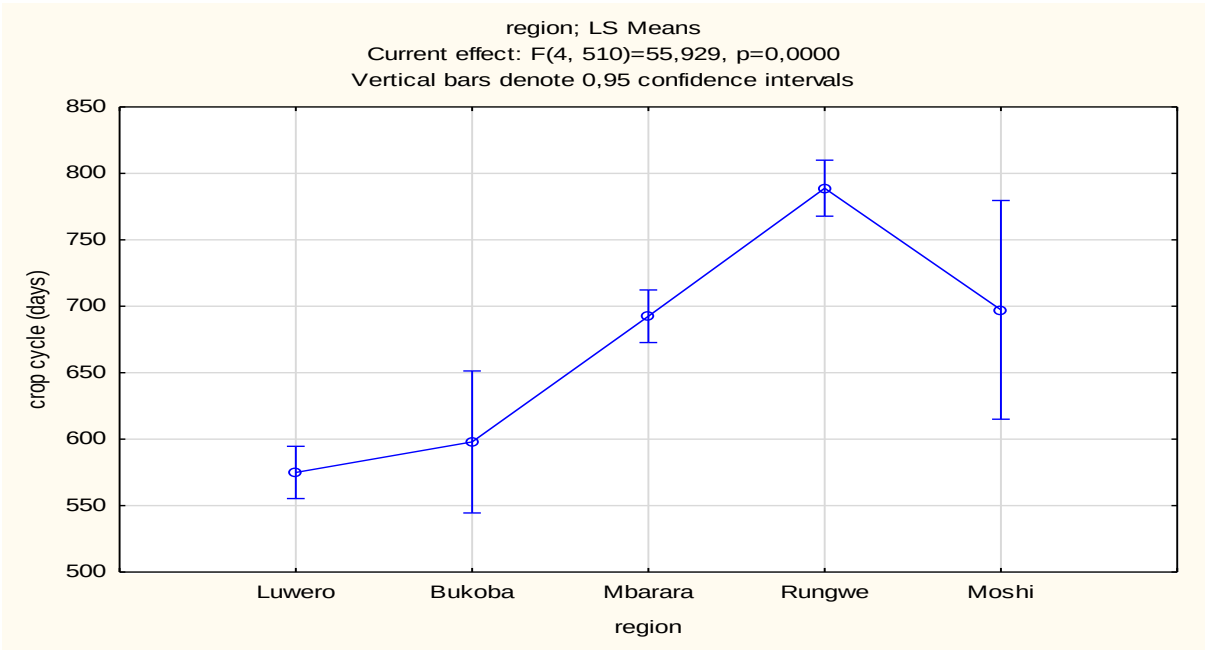

Figure 8: Average first crop cycle in days of the 3 cultivars Cachaco, Mbwazirume and Williams in the 5 different regions. N=20-229

So far we were able to analyse the data coming from 3 crop cycles in Luwero, in collaboration with the National Agricultural Research institute from Uganda NARO. Indeed Mbwazirume, the local cultivar, has an overall good vigorous yield during the first crop cycle reaching fast a cumulative yield of over 600 kg (Figure 9). As predicted, the cultivar Williams seems to cope well with the relatively lower temperatures and is the earliest yielding variety (Figure 9). Its earliness has as consequence that the cycle 1 yield starts in the dry period, and lasts into the second dry season, which slows down the yield to 500 kg. Cachaco has a less vigorous yield as predicted but the conservative strategy ensures also a cumulative yield of 500 Kg. On the long term at crop cycle 3, it has the highest yield (Figure 9). Mbwazirume was a preferred variety concerning quality characteristics in Luwero, Moshi, and Bukoba (Madalla et al in preparation). However, as demonstrated, it is quite sensitive to environmental stress which results in a steep drop in yield.

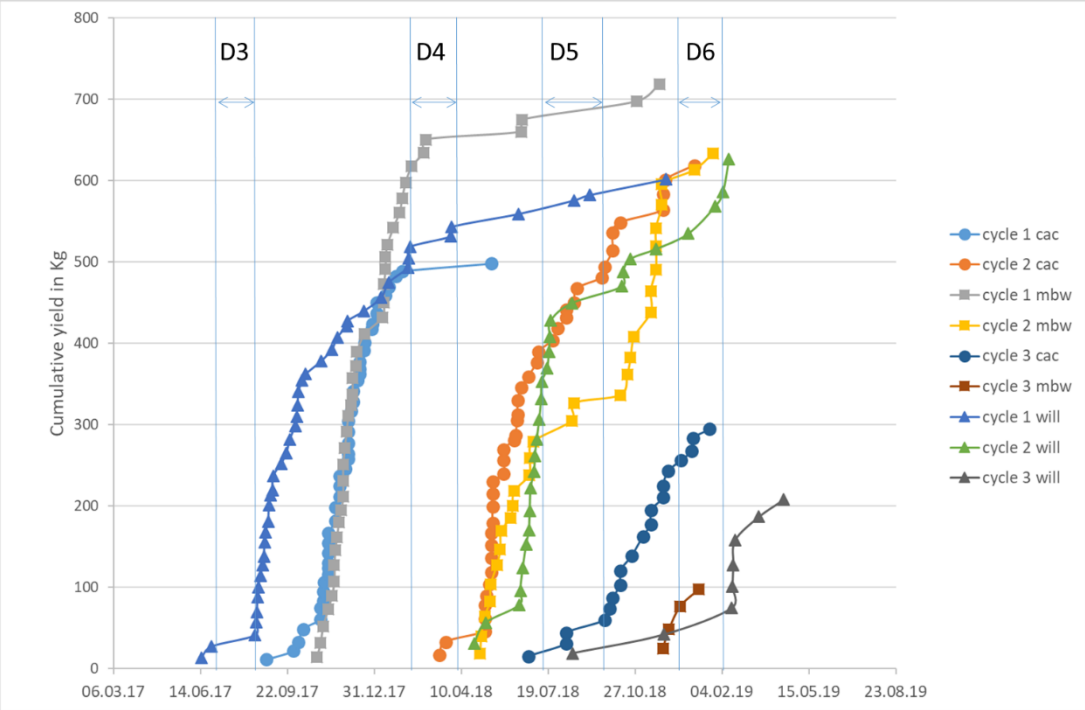

Figure 9: Impact of the different dry seasons on the yield. All cultivars were planted on May 5th 2016. The first bunch is harvested at the end of the wet season mid-June 2017. At that moment all plants haven been experiencing 2 dry seasons. Once the rainy season starts after dry season 3, the yield of all 3 investigated cultivars steeply increases. The impact of dry season on the first crop cycle (D4) is a delay in harvest especially in Mbwazirume (mbw), and Williams. The impact of the dry season on the second cycle (D5), the ratoon cycle, is substantial in Mbwazirume and Williams. The impact of the accumulation of 5 dry seasons is also visible in the third cycle of Cachaco (cac). Some plants die and need to be replaced, resulting in diminishing yield. The strategy of Cachaco to grow less vigorously and save water seems to work. Although the average bunch yield per plant is only 13.96 Kg versus 19.59 and 20.34 Kg for Williams and Mbwazirume respectively, the yield after 3 cycles is clearly better and has caught up the yield gap.

● WP4: Molecular analyses of drought tolerance[1]

With support from the Belgian government we have built up more than 30 years of expertise and infrastructure to unravel the untapped potential of banana diversity for current and future use. However, as long as gene banks are not fully characterized, phenotyped and accessible on a web platform such as MGIS (Ruas et al., 2017), it is difficult to comprehend what is missing in the germplasm collections. In the past DGD projects we have explored the diversity of our valuable collection by developing a phenotyping setup to study water related stress in banana under controlled environmental conditions (Carpentier, 2015; Carpentier et al., 2011; Kissel et al., 2015; Vanhove et al., 2012; Zorrilla et al., 2016). To explore the genetic diversity and the unique genes of the ABB varieties, an in-depth study was performed and confirmed that unique chromosome structures exist with unique alleles that divide the ABB subgroup in 9 different subdivisions (Cenci et al., 2020). We have additionally confirmed that those specific chromosome structures and unique alleles also have an impact on the gene expression and consequently on the final specific traits a plant has (Cenci et al., 2019). All unique gene sequences have been made available on our gene bank web portal https://www.crop-diversity.org/mgis/gigwa. Since the beginning of our project, one cultivar has been a reference, Cachaco belong to the Bluggoe group. Bluggoe is also locally grown in small amounts (Table 1). Therefore, the production of genome resources on the Cachaco genome is critical, in order to better understand the molecular mechanisms behind the different traits.

However, this is a challenging objective due to the nature of the cultivar which has a complex polyploid genome. The sequencing of plant genomes has accelerated over the past years but still, few polyploid plants were made available also in other crops. In this project, we have generated long read sequences using the novel Oxford Nanopore Technologies (https://nanoporetech.com/) to sequence the triploid Cachaco genome. This genome will serve as a reference genome for the whole ABB group. Reconstructing a correct assembly for a polyploid plant that originated from two different parent species is not an easy process. As this is the first time in banana, we decided to benchmark various bioinformatics tools. Lastly, two separate workflows were created to facilitate reproducibility and sharing of the methodology. A total of 10,5 GB of sequence reads were produced. We conducted an assembly guided strategy using both A and B genomes, which are representative of the ancestral species that led to the emergence of triploids. This first assembly is consistent with genome structure proposed for the A and B genomes (D'Hont et al., 2012; Wang et al., 2019). We could distinguish the two subgenomes. We also find in on our assembly evidence of a large translocation and an inversion between chromosomes A and B on chromosomes 4 and 11, where the A genome sequence was replaced the B genome counterpart. This also confirms our previous study (Cenci et al., 2020).

[1] This WP has suffered some delay due to COVID 19.

What makes individual cultivars unique so that they behave differently towards the same exposed environment? Do they contain unique genes that can be inherited? To answer those questions we have started to study the gene expression in the guard cells. Guard cells, are the cells controlling the stomatal opening and control many of the data we have been phenotyping. We have already delivered a proof of principle of guard cell isolation and analysis in the model plant Arabidopsis (Dittrich et al., 2019; Geilfus et al., 2018), and we have also proven that we can detect cultivar unique genes in banana (van Wesemael et al., 2018a). We successfully tested a novel protocol (Jalakas et al., 2017), where guard cells have been extracted through blending. We show that we are able to isolate guard cells in banana and extract the specific messengers and proteins when 9 different cultivars were exposed to different levels of dry air. At the driest air level we see clearly different phenotypes and different proteomes. Further analysis is in progress.

Another focus of this WP was the genome analysis of the aquaporin gene family. Aquaporin genes encode for proteins that are involved in water transport. Their regulation and variability in sequence have the potential to influence drought stress in plants (Johansson et al., 2000; Johansson et al., 1998; Maurel et al., 2015). They play a crucial role in stress responses by controlling water transport across membranes (Di Pietro et al., 2013; Sade et al., 2014). The banana genome has 20 genes coding for a plasma membrane aquaporin. Possibly due to their ancient origin and their essential function in cell life, protein sequences of aquaporins are very strongly conserved. However we did find some sequence divergence especially in loop A and C of the protein. In total 131 differences were mapped and further research is needed to determine causal relations between the water transport and the observed variability.

● WP5: Documentation, capacity building and outreach.

A key component throughout the project is the cross-cutting work-package WP5. The data resulted from this project have been integrated into the existing digital platforms MGIS and GWAS and the proteomics data repository PRIDE, to optimise public data access.

On 18 December 2018, we explained the concept of our research of WP1 the BananaTainer on prime-time national and regional television (VTM nieuws, ROB), and radio (Radio 2). Those reportages were subsequently picked up by many international magazines such as freshplaza.

We have made a video about our phenotyping work (WP2) on YouTube https://youtu.be/c65_69i8n6c , on the EPPN website https://eppn2020.plant-phenotyping.eu/Selected_Projects and a blog and have shared it on social media. (https://www.cwrdiversity.org/wild-about-bananas/; and on the Australian radio (Radio Sydney) https://www.abc.net.au/news/rural/2020-11-26/ancient-bananas-in-png-can-save-the-worlds-favourite-fruit/12917960?section=world

It caught the attention of various international news agencies and was translated into several languages:

https://phys.org/news/2020-11-wild-bananas-papua-guinea.html,

https://www.freshplaza.com/article/9266679/searching-for-wild-bananas-in-papua-new-guinea/,

https://arabicapost.net/in-search-of-wild-bananas-in-papua-new-guinea-2/

https://www.scientias.nl/s-werelds-favoriete-fruit-dreigt-het-onderspit-te-delven-maar-wetenschappers-laten-het-er-niet-bij-zitten/

https://www.repubblica.it/green-and-blue/2020/11/09/news/in_papua_nuova_guinea_i_cercatori_di_banane_perdute-273705200/

https://mundoagropecuario.com/a-la-caza-de-platanos-silvestres-en-papua-nueva-guinea/

We have published 23 scientific papers in peer reviewed journals or books: (Baurens et al., 2018; Bhuiyan et al., 2020; Brown et al., 2020; Campos et al., 2018; Carpentier, 2020; Cenci et al., 2019; Cenci et al., 2020; Costa et al., 2019; Dittrich et al., 2019; Eyland et al., 2020; Geilfus et al., 2018; Kallow et al., 2020; Marimo et al., 2020; Martin et al., 2017; Rouard, 2018; Rouard et al., 2018; Ruas et al., 2017; Sempéré et al., 2019; Van den houwe et al., 2020; van Wesemael et al., 2018a; van Wesemael et al., 2019; van Wesemael et al., 2018b; Wang et al., 2019)

We have trained 2 Bachelor students: Simon Costers (AP Antwerpen), and Kaat Hebbelink (REGA), 3 Master's students: Bill Smeets (KULeuven), Marlies Vanluchene (KULeuven), and Anaïs Louis (U Montpellier), 4 PhD students: Jelle van Wezemael KULeuven, David Eyland (KULeuven), Clara Gambart (KULeuven), and Noel Madalla (SLU)) and 2 trainees: Nadine Aschauer (University of Warwick) and Suke Wang (CAAS).

The project has synergies with the European projects EPPN 2020 Bananadyn, and LEAPAGRI Clismaban; the prebreeding Global TRUST project on the wild banana diversity in Papua New Guinea; and the Bill & Melinda Gates projects on Breeding Better Bananas and BBTV mitigation.

Ultimately the output will be our insight into the weaknesses of the local varieties and that alternative selected banana varieties from the gene bank will be available to the local seed systems, boosting the adoption by millions of banana growers together with the farm management advice. The opportunity to increase the diversity in the seed systems and add healthy and potent planting material to their portfolio will mitigate the risk of yield loss from unpredictable seasons. As illustrated, there is a tendency to have the yield waves dampened the higher the crop cycle goes (Figure 9) so in the future farmers will benefit from the renewal of the mats. The evaluation of improved hybrids, diploid cultivars and wild varieties that are fertile, is a strong pre-breeding programme. The further the genetic characterisation evolves, the more likely breeders will have the biotechnological tools to speed up the breeding process. Within and beyond the target regions, this project intends to increase banana

yields and livelihoods by delivering the outstanding 'climate smart' planting material to the local seed systems, and engage with NARS to train farmers in best practices for farm management. For this to happen, various stakeholders (namely breeders (e.g. IITA, NARO), agronomists, social scientists, seed producers, agro-input traders, extension workers etc.) will each play an important role in setting up local businesses for delivering the agro-eco zone specific cultivars to farmers. Our outcomes contribute to CGIAR system-level outcomes 1 (poverty reduction) and 2 (stronger food security), and to UNDP Sustainable Development Goals 1 (poverty reduction), 2 (less hunger), 12 (responsible production/consumption) and 13 (climate- action).

Output	Indicative Indicators	SDG Targets	Means of verification and publications
Output 1: Growth potential assessed of ITC gene bank acessions	✓ Ranking of the growth potential of 104 banana genotypes under simulated highland conditions and identification of 21 "climate smart" varieties.	1 End poverty in all its forms everywhere 2 End hunger, achieve food security and improved nutrition and promote sustainable agriculture 13 Take urgent action to combat climate change and its impacts	• van Wesemael, J., Swennen, R., Roux, N., Carpentier, S. (**2020**). The importance of the light spectrum in a high-throughput phenotyping lab concept: evaluating transpiration and biomass growth of different banana cultivars under different blue/red light ratios. Acta Horticulturae 13-20 doi: 10.17660/ActaHortic.2020.1272.2 • PhD thesis: van Wesemael, J., Swennen, R. (sup.), Carpentier, S. (sup.) (2019). Mining the Musa biodiversity for drought tolerance: allele discovery via integrated phenomics, proteomics and transcriptomics.
Output 2	✓ Product characteristics and cultivar preferences of male and female farmers	3 Ensure healthy lives and promote well-being for all at all ages 5 Achieve gender equality and empower all women and girls	• Marimo, P., Caron, C., Van den Bergh, I., Crichton, R., Weltzien, E., Ortiz, R., and Tumuhimbise, R. (2020). Gender and Trait Preferences for Banana Cultivation and Use in Sub-Saharan Africa: A Literature Review 1. Economic Botany, 1-16. • Marimo, P., Karamura, D., Tumuhimbise, R., Shimwela, M., Van den Bergh, I., Batte, M., Massawe, C., Okurut, A., Mbongo, D., and Crichton, R. (2019). Post-harvest use of banana

Output	Indicative Indicators	SDG Targets	Means of verification and publications
			in Uganda and Tanzania: Product characteristics and cultivar preferences of male and female farmers.
Output 3: Differential growth patterns studied in detail	✓ 32 varieties have been evaluated under osmotic stress conditions. The publication is available to breeders and researchers through open access (van Wesemael et al., 2019). ✓ 15 varieties have been phenotyped in detail on the high throughput phenotyping platform Phenodyn (Collaboration with INRA in EPPN 2020 EU project; Eyland et al in preparation). ✓ Stomatal conductance mechanisms of 5 varieties has been studied in detail and submitted for publication (Eyland et al submitted).	4 Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all	• van Wesemael, J., Kissel, E., Eyland, D., Lawson, T., Swennen, R., Carpentier, S. (2019). Using Growth and Transpiration Phenotyping Under Controlled Conditions to Select Water Efficient Banana Genotypes. FRONTIERS IN PLANT SCIENCE, 10, Art.No. ARTN 352. doi: 10.3389/fpls.2019.00352 • Eyland, D., Breton, C., Sardos, J., Kallow, S., Panis, B., Swennen, R., Paofa, J., Tardieu, F., Welcker, C., Janssens, S.B. and Carpentier, S.C. (**2020**), Filling the gaps in gene banks: Collecting, characterizing and phenotyping wild banana relatives of Papua new guinea. Crop Sci. doi:10.1002/csc2.20320
Output 4: Variety evaluation in the field and validation of lab models	✓ Field evaluation of 3 varieties at 5 locations (Tanzania and Uganda). (Machida et al in preparation)	2 End hunger, achieve food security and improved nutrition and promote sustainable agriculture 13 Take urgent action to combat climate change and its impacts	• Carpentier, S. Eyland, D. (**in press**) Genetic approaches to improve abiotic stress factors in banana. In: Achieving sustainable cultivation of bananas Vol 2: Germplasm and genetic improvement (ed. Prof Gert Kema & Prof André Drenth)

Output	Indicative Indicators	SDG Targets	Means of verification and publications
	✓ Field evaluation of 4 varieties in Tanzania for 2 crop cycles. (Uwimana et al submitted)		• Brown, A., Carpentier, S. C., & Swennen, R. (**2020**). Breeding Climate-Resilient Bananas. In Genomic Designing of Climate-Smart Fruit Crops (pp. 91-115). Springer, Cham. doi:10.1007/978-3-319-97946-5_4
Output 5: Molecular mechanisms behind drought tolerance analyzed	✓ From a sub-sample of 12 and 36 varieties respectively the molecular and genetic analysis have been published (Cenci et al., 2019; Cenci et al., 2020). List of candidate alleles have been selected, genome recombination signatures and chromosome losses have been detected. ✓ Researchers have open access to transcriptomic data publicly accessible in scientific databases.	2 End hunger, achieve food security and improved nutrition and promote sustainable agriculture 4 Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all 13 Take urgent action to combat climate change and its impacts	• Cenci, A., Hueber, Y., Zorrilla, J., van Wesemael, J., Kissel, E., Gislard, M., Sardos, M., Swennen, R., Roux, N., Carpentier, S.C., Rouard, M. (**2019**). Effect of paleopolyploidy and allopolyploidy on gene expression in banana. BMC GENOMICS, 20 (244), 1-12. doi: 10.1186/s12864-019-5618-0 • Cenci, A., Sardos, J., Hueber, Y., Martin, G., Breton, C., Roux, N., Carpentier, S., Rouard, M. (**2020**). Unravelling the complex story of intergenomic recombination in ABB allotriploid bananas. Annals of Botany. Doi: 10.1093/aob/mcaa032 van Wesemael, J., Hueber, Y., Kissel, E., Campos, N., Swennen, R., Carpentier, S. (2018). Homeolog expression analysis in an allotriploid non-model crop via integration of transcriptomics and proteomics. Scientific Reports, 8, Art.No. 1353. doi: 10.1038/s41598-018-19684-5 Carpentier, S. C. (2020). The Use of Proteomics in Search of Allele-Specific Proteins in (Allo) polyploid Crops. In Plant Proteomics (pp. 297-308). Humana, New York, NY.
Output 6: New knowledge shared with stakeholders	✓ Technical guidelines for lab and field phenotyping have been developed for local research	4 Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all	• PhD thesis Jelle van Wesemael: van Wesemael, J., Swennen, R. , Carpentier, S. (2019). Mining the Musa biodiversity for drought tolerance: allele discovery via integrated phenomics, proteomics and transcriptomics.

Output	Indicative Indicators	SDG Targets	Means of verification and publications
(breeders and researchers) in the Great Lake Area (i.e., generated knowledge feeds into banana breeding and planting material supply chain)	stations and will be promoted via Musanet. ✓ Publications and setup of the databases linked to MGIS[2] and Musabase[3]. ✓ PhD started: ○ David Eyland (BE): Crop wild relatives: wild banana the key to drought tolerance? ○ Clara Gambart (BE): The fundaments of crop growth: modelling growth and guard cell physiology in relation to light, vapor pressure deficit and soil water potential ○ Noel Madala (TZ): End-users' traits preferences for improved banana cultivars in Tanzania and Uganda		• Master thesis Bill Smeets: Smeets, Bill. Understanding Water Deficit Response in Bananas: Screening Beneficial Traits in Wild Relatives by Phenotyping Root Properties, Leaf Emergence and Transpiration Dynamics. Leuven: KU Leuven. Faculteit Bio-ingenieurswetenschappen, 2020. Web. • Carpentier, S., Iyyakutty, R.,Kissel, E.,van Wesemael, J. Tomekpe, K., Roux, N., Dita, M. (**in press**). Phenotyping protocol for drought tolerance in banana. Musanet

[2] https://www.crop-diversity.org/mgis/
[3] https://musabase.org/

Output	Indicative Indicators	SDG Targets	Means of verification and publications
	✓ Master dissertation: ○ MSc Marlies Vanluchene: 'Exploring nitrogen use efficiency of banana in the Bananatainer and the greenhouse' ○ MSc Bill Smeets: Understanding water deficit response in bananas ○ MSc Anaïs Louis: Evaluation of tools and strategy for the assembly of an allopolyploid banana genome		

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