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SPECIAL ISSUE: ADAPTING AGRICULTURE TO CLIMATE CHANGE: A WALK ON THE WILD SIDE

Filling the gaps in gene banks: Collecting, characterizing, and phenotyping wild banana relatives of Papua New Guinea

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Abstract

Since natural habitats are disappearing fast, there is an urgent need to collect, characterize, and phenotype banana (*Musa* spp.) crop wild relatives to identify unique genotypes with specific traits that fill the gaps in our gene banks. We report on a collection mission in Papua New Guinea carried out in 2019. Seed containing bunches were collected from *Musa peekelii* ssp. *angustigemma* (N.W.Simmonds) Argent (3), *M. schizocarpa* N. W. Simmonds (4), *M. balbisiana* Colla (3), *M. acuminata* ssp. *banksii* (F. Muell.) Simmonds (14), *M. boman* Argent (3), *M. ingens* Simmonds (2), *M. maclayi* ssp. *maclayi* F.Muell. ex Mikl.-Maclay (1), and *M. lolodensis* Cheesman (1). This material, together with the seeds collected during a previous mission in 2017, form the basis for the development of a wild banana seed bank. For characterization and phenotyping, we focused on the most ubiquitous indigenous species of Papua New Guinea: *M. acuminata* ssp. *banksii*, the ancestor of most edible bananas. We calculated that the median genomic dissimilarity of the *M. acuminata* ssp. *banksii* accessions was 4% and that they differed at least 5% from accessions present in the International Transit Centre, the world's largest banana gene bank. High-throughput phenotyping revealed drought avoidance strategies with significant differences

Abbreviations: CGIAR, Consortium of International Agricultural Research Centers; GBS, genotyping-by-sequencing; ITC, International Transit Centre; SNP, single nucleotide polymorphism; VPD, vapor pressure deficit.

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in root/shoot ratio, soil water content sensitivity, and response towards vapor pressure deficit (VPD). We deliver a proof of principle that the wild diversity is not yet fully covered in the gene banks and that wild *M. acuminata* ssp. *banksii* populations contain individuals with unique traits, useful for drought tolerance breeding programs.

1 | INTRODUCTION

Currently, there is often a lack of crop diversity in the fields, which increases the vulnerability to abiotic and biotic constraints. Moreover, we are being challenged by climate change, which brings alterations in temperature and precipitation and an increased frequency of drought and floods. To guarantee food security, more diversity needs to be introduced and new varieties with increased tolerance to abiotic and biotic factors need to be developed. Crop wild relatives, the wild progenitors of cultivated crops, can be a source of new allelic diversity (Hajjar & Hodgkin, 2007).

In situ and ex situ conservation programs have been set up in the framework of national and international conservation strategies. The 11 international gene banks of the Consortium of International Agricultural Research Centers (CGIAR) were placed under the auspices of the Food and Agriculture Organization (FAO) of the United Nations in 1994, with the mission to conserve germplasm collections for 22 mandate crops. Even though these are the most consulted ex situ collections, a large fraction of the managed germplasm remains neither characterized nor phenotyped. As long as gene banks are not fully characterized, phenotyped, and accessible on a web platform such as the Musa Germplasm Information System (MGIS; Ruas et al., 2017) or GERMINATE (Lee et al., 2005), it is difficult to comprehend what is missing in the germplasm collections. Moreover, the diversity of crop wild relatives is poorly represented in gene banks (Castañeda-Álvarez et al., 2016; Vincent et al., 2013). Unique traits and associated alleles can be identified through collecting, characterizing, and evaluating crop wild relatives (Dempewolf et al., 2017). In the case of bananas (*Musa* spp.), the CGIAR supported by the Global Crop Diversity Trust and KU Leuven have collected and managed the world banana heritage in the International Transit Centre (ITC) located in Belgium, with the aim to secure long-term conservation for future generations (Van den houwe, De Smet, du Montcel, & Swennen, 1995; Van den houwe, Lepoivre, Swennen, Frison, & Sharrock, 2003).

The wild banana species *Musa acuminata* Colla, *M. balbisiana* Colla, and to a small extent *M. schizocarpa* N.W. Simmonds and *M. textilis* Née are the main ancestors of the cultivated edible bananas (Carreel et al., 1994; Němečková et al., 2018; Šimoníková et al., 2019). In contrast with cultivated bananas that are parthenocarpic and mostly sterile, wild bananas produce seeds enabling them to perform sexual reproduction besides vegetative propagation. Similar to other crop wild relatives, wild bananas are under selection pressure in natural environments and evolutionarily developed some tolerance to biotic and/or abiotic constraints. Hence, the diversity of seeded bananas is crucial for banana breeding programs. Their inter- and intraspecific diversity is vital to identify useful alleles and to introduce genetic diversity into the narrow genetic basis of the edible bananas. Wild bananas are native to the humid tropical and subtropical forests of Southeast Asia and Near Oceania. These forests are rapidly destroyed (Filer, Keenan, Allen, & McAlpine, 2009; Mietteninen, Shi,

& Liew, 2011; Sodhi, Koh, Brook, & Ng, 2004), thereby threatening the diversity of wild banana species. However, none of the gene banks covers the wild banana diversity at a species level. A high priority for further collecting has been assigned to crop wild relatives of banana, indicating the urgent need for conservation (Castañeda-Álvarez et al., 2016). Even though seeds of some wild bananas have been collected in the past, there is no good knowledge of the population diversity, the micro-environment (habitat) of collected individuals, and their potential traits. This lack of knowledge raises practical questions, such as which core subsets of seeds are needed to maximize genetic distance and/or allelic richness. In a previous study, the genetic diversity of seven *M. balbisiana* seed collections was assessed and the highest genetic diversity was found in seeds from natural populations (Bawin et al., 2019). These results show that additional collection of seeds from natural populations, preferentially originating from the species' native distribution range, is crucial to fill the gaps and safely conserve the diversity of a species.

Eight wild banana species [*M. acuminata* ssp. *banksii* (F. Muell.) Simmonds, *M. boman* Argent, *M. bukensis* Argent, *M. ingens* Simmonds, *M. lolodensis* Cheesman, *M. maclayi* F.Muell. ex Mikl.-Maclay, *M. peekelii* Lauterb., and *M. schizocarpa*] are native to Papua New Guinea. *Musa balbisiana* occurs as well but was most likely introduced (Argent, 1976; Perrier et al., 2011).

Among the wild populations of Papua New Guinea, *M. acuminata* ssp. *banksii*, *M. schizocarpa*, *M. balbisiana*, and hybrids of the two former species are of direct interest for prebreeding, since they are more closely related to our current cultivars and are easier to introgress (Carreel et al., 1994; Christelová et al., 2017; Perrier et al., 2009; Šimoníková et al., 2019). Papua New Guinea has diverse agroecological conditions, varying from lowland to highlands, each with a different set of banana populations and under different selective pressures. Within Papua New Guinea, the most ubiquitous ancestor of the edible bananas is *M. acuminata* ssp. *banksii*, whose distribution extends to Australia and Samoa (Argent, 1976; Simmonds, 1956). *Musa schizocarpa* is somewhat less common than *M. acuminata* ssp. *banksii* but has an overlapping distribution range in Papua New Guinea (Argent, 1976; Arnaud & Horry, 1997; Sardos, Paofa, Janssens, Vanden Abeele, & Panis, 2017; Sharrock, 1989). *Musa balbisiana* is less adapted to the natural humid rainforest. It grows in areas of lower rainfall and occurs in Papua New Guinea in grasslands created by burning forests (Argent, 1976; Sharrock, Jones, & Banag, 1989).

A series of four banana collecting missions took place between 1988 and 1989 in Papua New Guinea (Sharrock, 1988, 1989; Sharrock, Daniells, & Kambuou, 1988; Sharrock et al., 1989). Only 39 wild individuals were collected and incorporated as a clonal accession in the ITC. None of them have been phenotyped for useful traits. It is clear that the 1980s collection missions to Papua New Guinea did not focus on wild banana species (Arnaud & Horry, 1997; Simmonds, 1956). The ITC is the world's largest banana gene bank and currently contains 1,617 accessions. However, the intraspecific diversity of the different wild banana species is largely underrepresented, as it encompasses only 31 *M. balbisiana*, 30 *M. acuminata* ssp. *banksii*, 16 *M. acuminata* ssp. *malaccensis* Ridl., 13 *M. schizocarpa*, eight natural hybrids between *M. acuminata* ssp. *banksii* and *M. schizocarpa*, six *M. acuminata* ssp. *zebrina* (Van Houtte ex Planch.), three *M. acuminata* ssp. *siamea* N.W. Simmonds, three *M. acuminata* ssp. *burmannica* N.W. Simmonds, one *M. acuminata* ssp. *truncata* (Ridl.), and one *M. acuminata* ssp. *microcarpa* (Becc.) N.W. Simmonds wild accessions. To reduce the underrepresentation of wild bananas in the ITC, new collection missions have been organized over the past 4 yr (Sachter-Smith, Paofa, Rauka, Sardos, & Janssens, 2017; Sardos et al., 2016, 2017). Here, we aimed to add value to the ITC gene bank by collecting new wild banana accessions, characterizing the genetic diversity, and evaluating important phenotypic traits. We report the outcome of a 2019 collection mission to Papua New Guinea and explore the genetic diversity within and across *M. acuminata* ssp. *banksii* populations. We deliver a proof of principle that the collected *M. acuminata* ssp. *banksii* differ in genome sequence from the individuals currently present in the ITC gene bank and show that the collected individuals display phenotypic diversity.

2 | MATERIALS AND METHODS

2.1 | Collection mission and documentation

A collecting trip to mainland Papua New Guinea took place from 12 until 25 May 2019. The team consisted of researchers from KU Leuven, Bioversity International, Meise Botanic Garden, and the National Agricultural Research Institute of Papua New Guinea (NARI). The aim was to collect novel wild banana diversity from diverse habitats. Three regions were visited: Morobe, Madang, and Sandaun provinces.

Wild banana bunches exhibiting developed fruits, preferentially with the oldest fruits showing some level of maturity, were considered. Seeds were assessed for maturity based on their hardness, color, and the presence of an embryo, and only bunches containing mature seeds were collected. The parent plant was described morphologically, and a leaf sample from the parent or sucker was taken for DNA extraction. Each accession was labeled

with a unique collection code, GPS coordinates, and local habitat observations. Fruits were removed from the bunch and transferred to paper bags according to their hand number. Seeds remained in the fruit during transport and were extracted and dried for storage upon arrival in Belgium (2–4 wk). In view of the erratic and low germination rates of wild banana seed in soil, embryo rescue was performed to regenerate the maximal number of individuals. QGIS software (version 3.0.2) was used to calculate the altitude of each accession, the distances between accessions and to create maps. Altitudes were quantified by interpolating coordinates on the digital elevation model of the NASA Shuttle Radar Topography Mission (SRTM).

2.2 | Genotyping

From each local *M. acuminata* ssp. *banksii* population visited by Sardos et al. (2017) in the Madang and Morobe provinces of Papua New Guinea one individual was randomly selected for characterization of the leaf samples via DarTseq (in total, eight). These eight individuals were compared to eight ITC accessions of the same taxon that were analyzed via genotyping-by- sequencing (GBS) by Sardos et al. (2016). DarTseq sequences were processed by the Diversity Arrays Technology company, Australia (Kilian et al., 2012). Between 1,883,540 and 2,456,269 bp single-end reads were obtained per biological sample. Read quality was checked via FastQC (Andrews, 2010). Reads were then cleaned to remove illumina adapter sequences and low-quality ends (Phred score > 20) with Cutadapt (Martin et al., 2016). After trimming, reads <30 bp were discarded. Reads were then aligned against the *Musa acuminata* genome of reference version 2 (DH Pahang; Martin et al., 2016), together with the GBS sequences using Burrows–Wheeler aligner's Smith–Waterman alignment (BWA-MEM; Li & Durbin, 2010). Realignment was done with module IndelRealigner, and the single nucleotide polymorphism (SNP) calling with the module HaplotypeCaller of GATK version 4.1 (McKenna et al., 2010). The HaplotypeCaller is able to call SNPs and insertion–deletions (indels) simultaneously via local de novo assembly of haplotypes in an active region. In order to take into account interspecies variation, the SNP calling was done on all samples simultaneously. All the bioinformatic analyses were processed on the South-green platform. The pipeline used to perform that analysis is available at https://github.com/CathyBreton/Genomic_Evolution. A dissimilarity matrix was calculated according to the Sokal and Michener index (Sokal & Michener, 1958). Accession heterozygosity was calculated as the number of heterozygous loci divided by the total number of loci. A neighbor-joining tree based on the dissimilarity matrix was constructed with the R package Ape (Paradis, Claude, & Strimmer, 2004).

2.3 | Phenotyping

Seeds from two *M. acuminata* ssp. *banksii* genotypes collected in Morobe and Madang province of Papua New Guinea by Sardos et al. (2017) were embryo rescued following Bawin et al. (2019) (Supplemental Figure S1). Two seedlings were randomly selected: one from the parent accession *SJP416* in Madang province which was called *banksii.11*, and one from the parent accession *SJP814* collected in Morobe province which was called *banksii.17*. The seedlings were cloned following Vuylsteke (1989) until at least 96 replicates were available for phenotyping (Supplemental Figure S1). The in vitro plants were acclimated at the Phenodyn phenotyping platform (Sadok et al., 2007) hosted at M3P, Montpellier Plant Phenotyping Platforms (https://www6.montpellier.inra.fr/lepse/M3P), Institut national de la recherche agronomique (INRAE), France, for at least 4 wk, after which they were phenotyped. Two experiments, one of 4 wk and one of 5 wk, respectively, took place in different seasons (spring [April–May] and fall [October–November]) to obtain full coverage of potential light and vapor pressure deficit (VPD) conditions. Air temperature, relative humidity, and light intensity were measured every 15 min at nine points in the greenhouse. Greenhouse temperatures were maintained at 26 ± 2 °C during the day and 20 ± 3 °C during the night. The photoperiod was set to 14 h. Additional light (200 μmol m^{-2} s^{-1}) was provided when external solar radiation was below 300 W m^{-2} by using 400 W HPS Plantastar lamps. The Phenodyn phenotyping platform is a greenhouse compartment containing high-precision lysimeters. These lysimeters provide a total weight measurement every 15 min, which was broken down into transpiration rate by correcting for plastic pot weight, soil weight, estimated plant weight, and soil evaporation. The plastic pot and tray were fixed weights, measured before the experiment. The soil dry weight of each pot was calculated by weighing the soil when filling the pots and taking a representative dry weight sample. The daily plant weight and leaf area were estimated from 13 red–green–blue images (12 side views from 30° rotational difference and one top view) taken twice a week at the Phenoarch phenotyping platform (Cabrera-Bosquet et al., 2016). Image-based parameters were correlated to leaf area and plant weight (R^2 > 96%). Plant biomass and leaf area were modeled by a power law equation over time (Paine et al., 2012). Soil evaporation was calculated using six pots on balances carrying no plant, but an artificial plastic plant mimicking the canopy

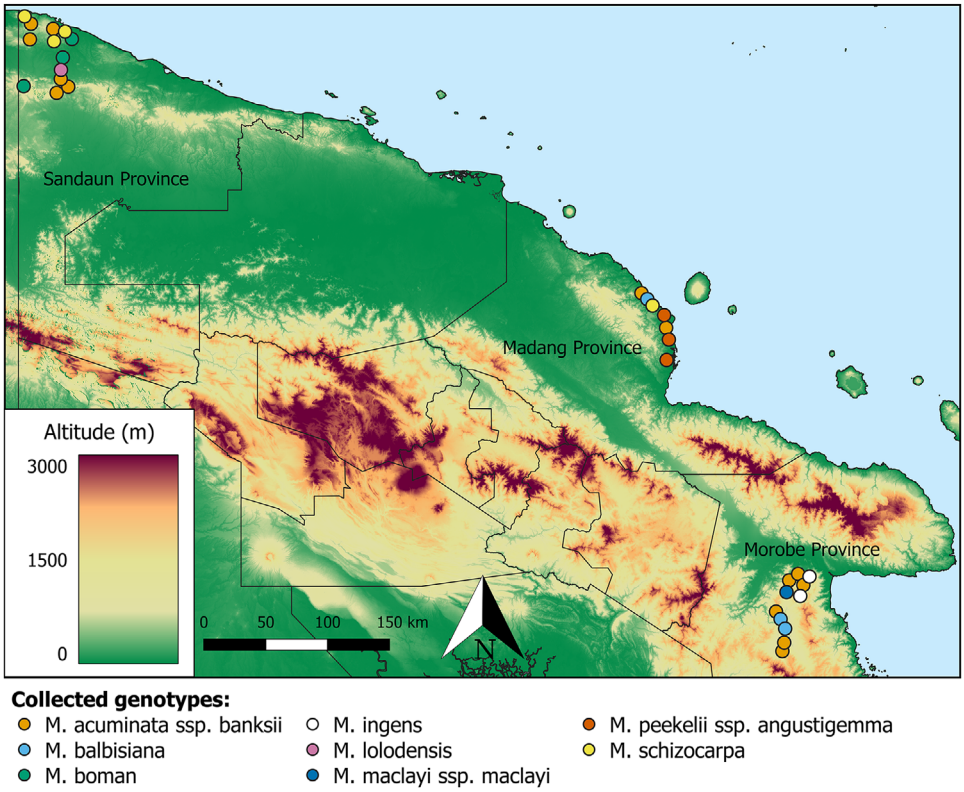


FIGURE 1 Sampling location of wild banana bunches in Papua New Guinea during the collection mission in 2019. Three provinces were visited: Morobe, Madang, and Sandaun. In total 31 different bunches were collected from nine different *Musa* species. Altitudes were derived from the digital elevation model of the NASA Shuttle Radar Topography Mission (SRTM)

soil cover. Daily transpiration rate was calculated by subtracting the daily soil evaporation from the total water loss during the daylight hours. Plants were grown in white 9 L pots filled with a well-mixed 30:70 (v/v) mixture of fine clay and organic compost. In the spring experiment, 16 *banksii.17* and 20 *banksii.11* plants were screened. In the fall experiment, 16 plants of both accessions were evaluated. In each experiment, half of the plants received a water-deficit treatment, whereas the other half was maintained under well-watered conditions. Genotypes and treatments were arranged in a randomized complete block design. Plants were irrigated at night when weight dropped below their target weight. The gravimetric water content of five 100 cm^3 soil samples was measured at two water potentials by the pressure plate method (Richards, 1948). Well-watered plants were maintained at field capacity (gravimetric water content of 1.40 g g^{-1}; soil water potential > −0.00631 MPa). The soil water content of water-deficit plants was allowed to decrease step-wise with a lower limit of −0.063 MPa (gravimetric water content of 0.85 g g^{-1}). To calculate the daily transpiration rate per unit leaf area, well-watered and water-deficit plants were maintained at a constant gravimetric soil water content of 1.40 and 0.95 g g^{-1}, respectively, during four consecutive days. To calculate the mean midday transpiration rate per unit leaf area, the threshold between low and high atmospheric VPD was defined at 1.7 kPa. At the end of the experiment, below- and aboveground biomass were weighted.

All data processing and subsequent statistical analyses were carried out in R (version 3.6.2). Two-way ANOVA was performed to test for significance between accessions. Linear mixed-effects models were used to test the difference between treatments and/or accessions with respect to repeated measurements.

Where possible, data were presented as box plots. The bold middle line in boxplots represents the median. The box is confined by the first and third quartile and the whiskers extend to 1.5 times the interquartile distance. Points falling outside the whiskers are considered as outliers.

3 | RESULTS AND DISCUSSION

3.1 | Collection and documentation

A total of 31 bunches of eight different species were collected covering a wide geographic range (Figure 1, Supplemental Table S1). We collected bunches of all *Musa* species previously described to occur on Papua New Guinea's

mainland (Argent, 1976; Arnaud & Horry, 1997; Sardos et al., 2017; Sharrock, 1988, 1989; Sharrock et al., 1988, 1989). Seed containing bunches were collected from *Musa peekelii* ssp. *angustigemma* (N.W.Simmonds) Argent (3), *M. schizocarpa* (4), *M. balbisiana* (3), *M. acuminata* ssp. *banksii* (14), *M. boman* (3), *M. ingens* (2), *M. maclayi* ssp. *maclayi* F. Muell. ex Mikl.-Maclay (1) and *M. lolodensis* (1).

The most abundant wild banana species in Papua New Guinea is *M. acuminata* ssp. *banksii*. During the field mission, the species was observed in Madang, Morobe, and Sandaun provinces under a variety of environmental conditions and an altitude ranging from sea level to 1,340 m asl (Supplemental Table S1). The color of its male bud ranged from green to yellow, red, or purple (Supplemental Figure S2). Similar observations were made by Argent (1976) and Sardos et al. (2017).

We found *M. balbisiana* between 18 and 593 m asl, almost always in open habitats where no other banana species occurred (Supplemental Figure S3). The other *Musa* species were always seen in small open patches or grew along roads through the humid tropical rainforest. The temperature and associated evaporative demand in open habitats can be very high, and temperatures up to 65 °C have been measured in full sunlight (McAlpine, Keig, & Falls, 1983). For a water-deficit-sensitive plant such as banana, survival under these conditions would only be possible provided there is efficient water uptake by the root system, a high root/shoot ratio, reduced stomatal conductance, and/or high temperature tolerance. It has indeed already been reported by Simmonds (1962) that *M. balbisiana* can withstand weeks of drought. This is also demonstrated by its origin in the monsoon region from West India to Southeast China (Janssens et al., 2016; Perrier et al., 2011). Moreover, in the edible bananas, the *M. balbisiana* B genome has been associated with improved tolerance to water deficit (Kissel, van Asten, Swennen, Lorenzen, & Carpentier, 2015; Thomas, Turner, & Eamus, 1998; van Wesemael et al., 2018, 2019; Vanhove, Vermaelen, Panis, Swennen, & Carpentier, 2012). Remarkably, large parts of *M. balbisiana* populations are located in open areas, but always in the proximity of water (Supplemental Figure S3) (Argent, 1976; Simmonds, 1956). Therefore, it can be hypothesized that *M. balbisiana* has more developed root systems that facilitate water uptake, allowing the plant to better cope with high evaporative demands and temperatures (Chaves et al., 2016). van Wesemael et al. (2019) already showed that cultivars with a high portion of *M. balbisiana* genes were characterized by a greater investment in root growth compared with shoot growth. Seedlings of *M. balbisiana* may possibly be outcompeted by other species under conditions with lower evaporative demand, such as small open patches in the tropical rainforests.

Conversely, *M. ingens* was reported by Argent (1976) in the rainforest at altitudes above 1,000 m asl. We found it at high altitudes of 1,350–1,450 m asl within a humid rainforest of the Morobe province. *Musa ingens* is the tallest banana species, reaching up to 15 m. This high stature enables it to compete for sunlight with surrounding trees (Supplemental Figure S4). This however would imply a higher hydraulic resistance (Domec et al., 2019) as water is transported over a huge distance. As a result, it is reasonable to assume that *M. ingens* is restricted to high altitudes because the low temperature, frequent fogs, and low evaporative demands create the conditions to transport water 15 m upwards without cavitating its xylem vessels. At low altitudes and associated higher temperatures, *M. ingens* was shown not to grow well, and the species seemed intolerant of continuous high temperatures (Argent, 1976). In order to reach sunlight at the top of the canopy, *M. ingens* seedlings must endure long periods of shade under the canopy cover. Hence, *M. ingens* is relatively shade tolerant, in contrast with other banana subspecies that suffer under such low-light conditions (Simmonds, 1962).

Musa schizocarpa co-occurs with *M. acuminata* ssp. *banksii*. *Musa schizocarpa* occurred in all three provinces between 0 and 429 m asl (Supplemental Table S1) but was less common than *M. acuminata* ssp. *banksii*. In addition, natural hybrids between *M. schizocarpa* and *M. acuminata* ssp. *banksii* were observed, showing intermediate features between both species. Observations of *M. peekelii* ssp. *angustigemma* were restricted to the Morobe province, whereas *M. maclayi* ssp. *maclayi* was only found in Madang province. A similar separation was observed by Argent (1976) and Sardos et al. (2017).

Musa lolodensis and *M. boman* were only found in Sandaun province. Both species were collected in a recently logged forest that was converted to an oil palm (*Elaeis guineensis* Jacq.) plantation. Large areas of rainforest are being deforested for these plantations resulting in habitat loss of *M. lolodensis* and *M. boman* in the region.

3.2 | Genomic diversity

Regarding genomic characterization, we focus on the most ubiquitous indigenous species, *M. acuminata* ssp. *banksii*. To estimate the gap in the gene banks, we compared accessions collected in Madang and Morobe provinces of Papua New Guinea with accessions present in the ITC gene bank (Table 1). The average distance between the accessions from Morobe and Madang province was 200 km and altitudes ranged from sea level to 665 m asl (Figure 2). The neighbor-joining tree in Figure 3 illustrates the diversity of the collected samples compared with *M. acuminata* ssp. *banksii* accessions present in the ITC gene bank (Table 1).

TABLE 1 List of the 16 *Musa acuminata* ssp. *banksii* accessions used in the genomic characterization. Eight accessions sampled in Papua New Guinea (Madang and Morobe province) were compared with eight accessions from the International Transit Centre (ITC). Their exact collection site, classification according to Sardos et al. (2016), and heterozygosity within the screened single nucleotide polymorphisms (SNPs) are shown

Sample code	Origin	Latitude	Longitude	Classification (Sardos et al., 2016)	Heterozygosity
ITC0378	Indonesia (Papua)	01°00′20.52″ S	134°48′36.36″ E	admixed	0.36
ITC0608	Australia (Queensland)	NA[a]	NA	admixed	0.21
ITC0896	Papua New Guinea (Manus island)	02°00′59.75″ S	147°15′00.00″ E	admixed	0.21
ITC0530	NA	NA	NA	admixed	0.19
ITC0602	Papua New Guinea (East Sepik province)	03°27′59.76″ S	143°28′59.88″ E	*M. acuminata* ssp. *banksii*	0.07
ITC0897	Papua New Guinea (Manus island)	02°00′59.75″ S	147°15′00.00″ E	*M. acuminata* ssp. *banksii*	0.03
ITC0620	Papua New Guinea (Madang province)	05°13′59.87″ S	145°45′00.00″ E	*M. acuminata* ssp. *banksii*	0.02
SJP416	Papua New Guinea (Madang province)	05°37′08.39″ S	145°28′06.60″ E	NA	0.02
SJP494	Papua New Guinea (Madang province)	05°29′16.22″ S	145°29′03.83″ E	NA	0.02
SJP504	Papua New Guinea (Madang province)	05°24′16.16″ S	145°38′02.39″ E	NA	0.02
SJP796	Papua New Guinea (Morobe province)	06°44′42.14″ S	146°43′58.80″ E	NA	0.02
SJP873	Papua New Guinea (Morobe province)	06°43′52.17″ S	146°43′54.11″ E	NA	0.02
SJP892	Papua New Guinea (Morobe province)	06°51′30.70″ S	146°36′48.24″ E	NA	0.02
ITC0606	Indonesia (Papua)	04°02′53.15″ S	138°08′44.88″ E	NA	0.01
SJP907	Papua New Guinea (Morobe province)	06°45′52.05″ S	146°38′52.79″ E	NA	0.01
SJP451	Papua New Guinea (Madang province)	05°34′30.10″ S	145°26′52.08″ E	NA	0.00

[a] NA, not applicable.

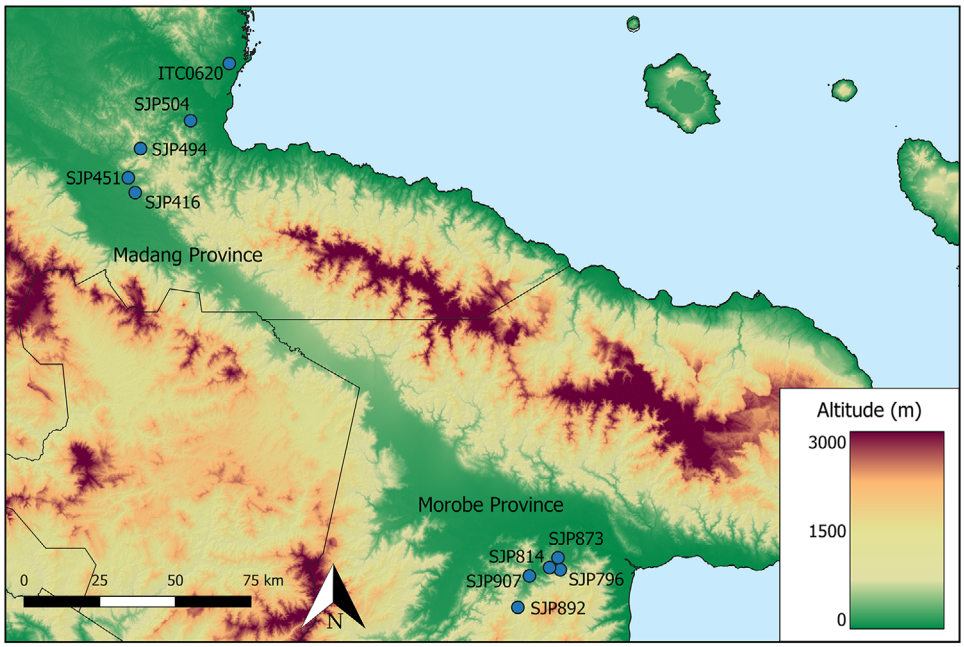


FIGURE 2 Sampling location of *Musa acuminata* ssp. *banksii* from Madang and Morobe province. The average distance between accessions collected in Madang and Morobe province was 200 km. The altitudes of sampled accessions ranged between 0 and 665 m asl. Altitudes were derived from the digital elevation model of the NASA Shuttle Radar Topography Mission (SRTM)

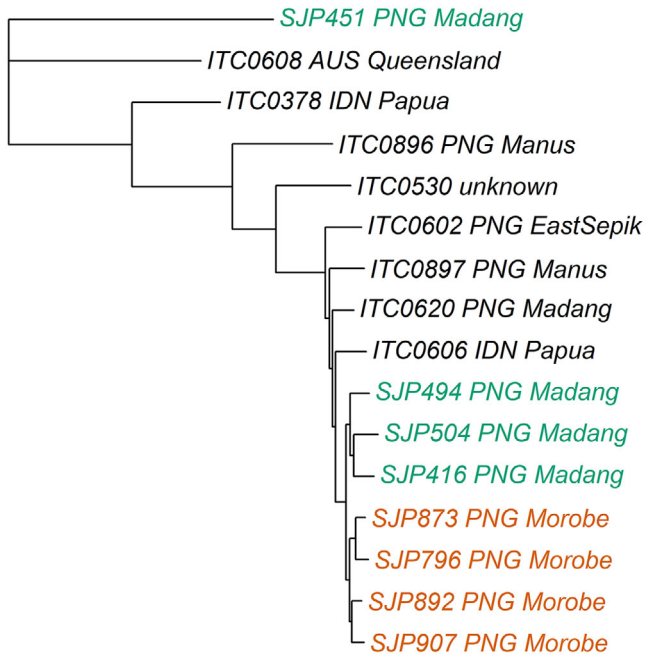


FIGURE 3 Neighbor-joining tree based on the dissimilarity across eight accessions of *Musa acuminata* ssp. *banksii* sampled in Papua New Guinea and eight accessions from the International Transit Centre (ITC). The first part of the labels indicates the accession code within the ITC gene bank or the collection code from the collection mission. The second and third part indicate the country and the province of origin. Colors indicate samples collected in Madang (green) or Morobe (orange). PNG, Papua New Guinea; AUS, Australia; IDN, Indonesia

The dissimilarity within the collected individuals of Morobe was, on average, 3% (Figure 4). Likewise, three out of four samples collected in Madang clustered together with 4% dissimilarity between the individuals. Across these two provinces, the mean difference was 4%, indicating that the intrapopulation diversity at these two collection sites was comparable with their interpopulation diversity. One individual sampled in Madang (*SJP451*) differed strongly, with 57% dissimilarity to the other collected *M. acuminata* ssp. *banksii* samples (Figures 3 and 4). Within the subset from the ITC collection, there were four accessions differing between 5 and 7% from the sampled Madang and Morobe populations (Figure 4). Other ITC accessions differed more from the sampled populations, with dissimilarities ranging between 15 and 30%. The ITC accessions only had, on average, 50% similarity with the deviating sample collected in Madang (*SJP451*) (Figure 4).

Except for a few accessions in the ITC collection that are admixed *M. acuminata* ssp. *banksii* species (Sardos et al., 2016), the levels of heterozygosity were very low (median = 2%; Table 1). Similar low heterozygosity levels were observed in *M. acuminata* ssp. *banksii* accessions by Sardos et al. (2016) and Martin et al. (2020), whereas admixed accessions showed higher heterozygosity. The low heterozygosity could be the result of frequent self-pollinating, which is believed to be common for *M. acuminata* ssp. *banksii*, as they contain ample hermaphrodite flowers (Argent, 1976; Simmonds, 1962). Simmonds (1962) hypothesized that close inbreeding could

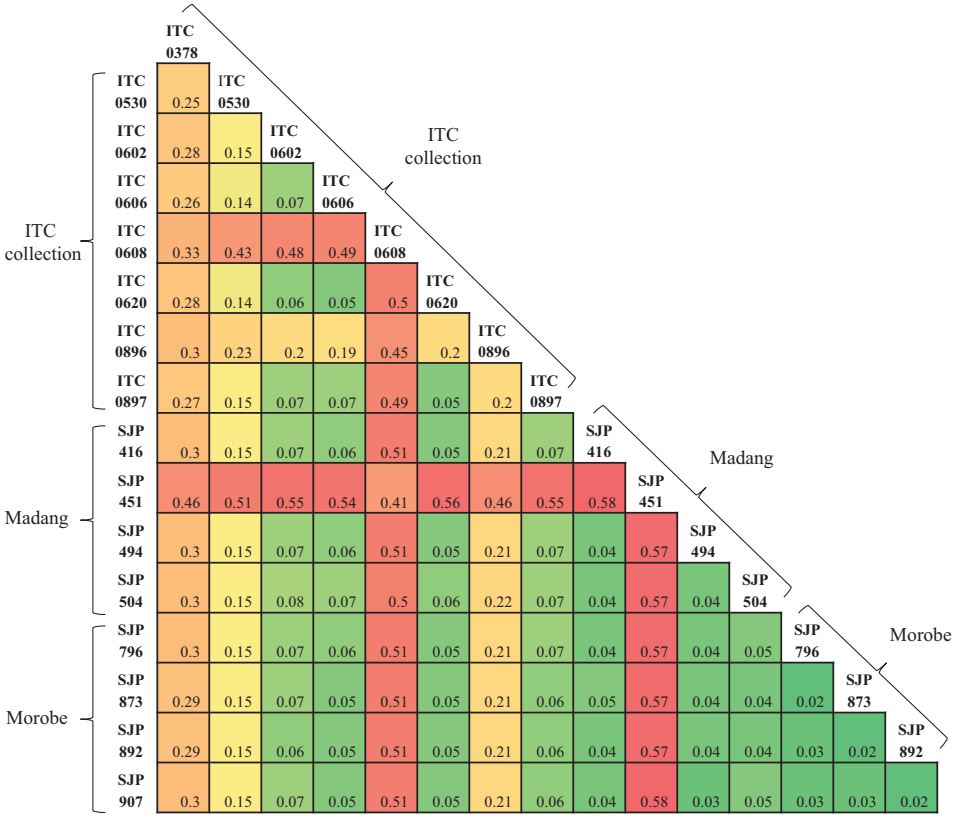


FIGURE 4 Dissimilarity matrix of eight accessions of *Musa acuminata* ssp. *banksii* sampled in Papua New Guinea (Madang and Morobe province) and eight accessions from the International Transit Centre (ITC) screened for single nucleotide polymorphism (SNP) markers. Values were calculated according to Sokal and Michener (1958). The color scale indicates minimal dissimilarities in green and maximal dissimilarities in red

also be the result of growing in regions physically isolated from other *M. acuminata* species, which is the case in Papua New Guinea.

In conclusion, with a median dissimilarity of 4%, the variability within the collected *M. acuminata* ssp. *banksii* samples was rather low (Figure 4), which may be caused by frequent self-pollination and close inbreeding. However, the collected material differed at least 5% from the *M. acuminata* ssp. *banksii* accessions currently in the ITC gene bank (Figure 4), indicating that new material was collected with unique alleles. The low variability combined with unique traits might lead to the easy identification of quantitative trait loci (QTLs). These results indicate that the wild diversity is not yet fully covered, and additional wild banana accessions need to be collected, characterized, and evaluated for specific traits and habitats.

3.3 | Phenotypic diversity

Two *M. acuminata* ssp. *banksii* seedlings, *banksii.11* from Madang and *banksii.17* from Morobe, were vegetatively multiplied and phenotyped to calculate the transpiration responses in function of soil water content and air VPD.

3.3.1 | Contrasting investment in root/shoot biomass

The two *M. acuminata* ssp. *banksii* accessions *banksii.11* and *banksii.17* differed in their investment in above- and belowground mass. This resulted in different root/shoot ratios (Figure 5). We hypothesize that this greater root/shoot investment is a drought avoidance strategy, as more water can be taken up in proportion to a smaller leaf area for transpiration.

3.3.2 | Contrasting soil water content sensitivity

Under well-watered conditions, the transpiration rate per unit leaf area was significantly higher in *banksii.11* than in *banksii.17* (Figure 6). Both accessions reduced water loss under water deficit, but the transpiration rate of

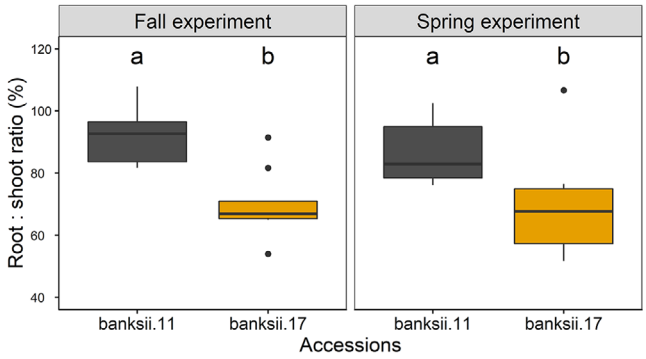


FIGURE 5 Different fresh weight root/shoot ratio between the plants of two *Musa acuminata* ssp. *banksii* accessions (*banksii.11* and *banksii.17*) at the end of the fall and spring experiments (n = 8–10). No common letter (a > b) indicates significant differences with p values < .05

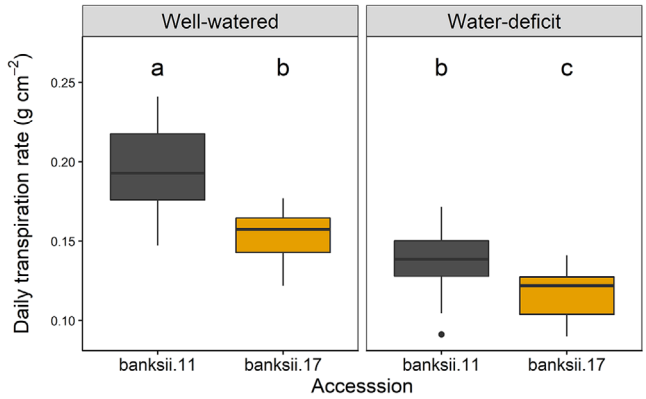


FIGURE 6 Daily transpiration rate per unit leaf area of two *Musa acuminata* ssp. *banksii* accessions (*banksii.11* in gray and *banksii.17* in orange) over four consecutive days (n = 8–10). Well-watered and water deficit plants were maintained at a constant gravimetric soil water content of 1.40 and 0.95 g g^{-1}, respectively. No common letter (a > b > c) indicates significant differences with p values < .05

banksii.11 remained significantly higher (Figure 6). The higher transpiration rate per unit leaf area of *banksii.11* under both well-watered and water deficit conditions is consistent with its higher root/shoot ratio (Figure 5). However, the transpiration rate reduction between well-watered and water deficit conditions was significantly higher in *banksii.11* than in *banksii.17* (p < .01). This reduction suggests a stronger stomatal control in *banksii.11* with decreasing soil water content.

3.3.3 | Contrasting VPD sensitivity under high soil water content

Plants adapt stomatal aperture not only in response to the available water in the soil but also in response to the

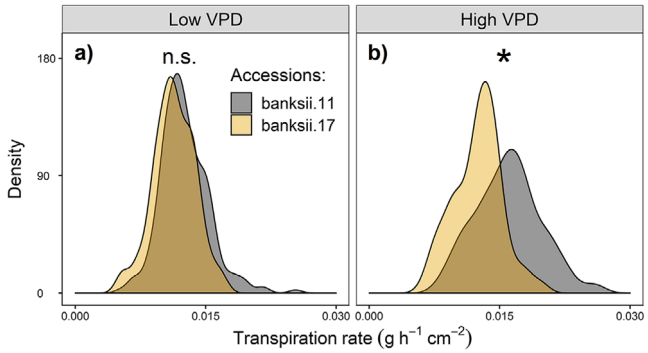


FIGURE 7 Mean midday transpiration rate per unit leaf area of the two *Musa acuminata* ssp. *banksii* accessions (*banksii.11* in gray and *banksii.17* in orange) under (a) low and (b) high atmospheric vapor pressure deficit (VPD). The y axis represents the kernel density estimate. The VPD threshold was defined as 1.7 kPa (n.s. for not significant, *p < .05)

atmospheric VPD. To investigate the influence of only the atmospheric VPD, the transpiration rate responses of both *M. acuminata* ssp. *banksii* accessions were compared under well-watered conditions. The transpiration rate per unit leaf area during the midday hours was similar for both accessions on days with low evaporative demand (mean VPD < 1.7 kPa, Figure 7a). During days with high evaporative demand (mean VPD > 1.7 kPa), the transpiration rate per unit leaf area of the *banksii.11* accession was significantly higher than that of *banksii.17* (Figure 7b). The accession *banksii.11* maintains a higher transpiration rate under high evaporative demands, a trait that has been linked to a higher leaf and root hydraulic conductance (Ocheltree, Nippert, & Prasad, 2014; Sadok & Sinclair, 2010). The higher root/shoot ratio of this accession (Figure 5) might improve the absolute water extraction in relation to its aboveground mass, enabling it to maintain its leaf water potential above a critical level and resist higher evaporative demands. This suggests that when the soil water content is nonlimiting, *banksii.11* maintains a higher stomatal conductivity under high VPD and thereby could maintain higher carbon fixation rates for a longer time than *banksii.17*.

To conclude, *banksii.11* shows a typical drought avoidance strategy with a higher root/shoot ratio, a better water transport capacity illustrated by the higher transpiration rate (also under high VPD), and a stronger stomatal control with decreasing soil water content. These characteristics indicate that *banksii.11* would be better adapted than *banksii.17* to environments where high evaporative demands occur and/or a short drought spell. However, this does not mean that *banksii.11* is able to thrive in an environment with a long-term drought period. A maintained transpiration rate under high VPDs and low soil water potential could result in a faster depletion of the available

soil water (Gholipoor, Prasad, Mutava, & Sinclair, 2010; Halperin, Gebremedhin, Wallach, & Moshelion, 2017; Tardieu, Draye, & Javaux, 2017).

We deliver a proof of principle of the existing diversity of water use traits within the wild banana subspecies *M. acuminata* ssp. *banksii*. Differences in transpiration rate under water deficit and high VPDs were discovered. We also show that within the *acuminata* genome, potential traits linked to improved water use efficiency exist. For breeding purposes, the usefulness of these phenotyped traits depends on the projected water deficit scenario (Passioura, 2012; Tardieu, 2012).

AUTHOR CONTRIBUTIONS

D.E., S.B.J., B.P., J.P., and S.C.C. performed the collection mission to Papua New Guinea. S.C.C. took photos during the collection mission. S.K., J.S., and S.B.J. designed the genomic analysis. S.K. extracted the DNA and prepared the samples for sequencing. C.B. and J.S. processed the genomic data. C.B., J.S., D.E., and S.C.C. analyzed the genomic data. D.E., C.W., F.T., and S.C.C. planned and designed the phenotyping experiments. D.E. processed the phenotyping data and made the region maps. D.E. and S.C.C. analyzed the phenotyping data. R.S. and S.C.C. supervised the experiments. D.E. and S.C.C. wrote the manuscript. All authors reviewed and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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