Strategies to revise agrosystems and breeding for Fusarium wilt control of banana

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Abstract

The recent emergence of the fungus *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4), the deadly strain that causes Fusarium wilt of bananas, has put the banana production chain for export under threat. Here, we propose research priorities and complementary strategies and challenges for an effective and efficient mitigation management of Fusarium wilt. Our strategies include biodiversing the agrosystems to increase crop resilience, as well as using precision breeding approaches to rapidly assess and introduce disease resistance genes to develop stable and complete *Foc* resistance in commercial banana cultivars.

Main

The recent identification of the fungus *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (*Foc* TR4), the most destructive and uncontrollable soil pathogen of banana (*Musa* spp.), in Colombia[1] is sending a dreadful message to the export plantations of Central and South America, demonstrating that this disease has become a global threat.

In (sub)tropical regions, banana production is significantly contributing to food security, as cooking bananas and plantains are considered staple crops in more than 120 countries around the (sub)tropical belt. The dessert banana 'Cavendish', which is internationally traded, is an important export commodity. Over the last fifty years, the average yield of banana in the world has nearly doubled, increasing from 10.6 tons/ha in 1961 to 20.2 tons/ha in 2017 (http://faostat.fao.org/). While the yield gain has been attributed to several factors, including climate change[2], the increasing acreage of banana cropped under intensive monoculture systems has significantly contributed to the increment in banana production.

Bananas originated from South(east) Asia and most of the domesticated varieties are seedless triploids (2n=3x=33) developed from specific (inter- and intra-) hybridizations of two wild diploid *Musa* species (*M. acuminata* and *M. balbisiana*)[3]. Modern edible varieties display genome constitutions of AAA (i.e. the cross of three *M. acuminata* genomes), as the sweet dessert and East African Highland bananas; AAB (cross of two *M. acuminata* and one *M. balbisiana* genomes), like starchy plantains and some dessert bananas; or ABB (cross of one *M. acuminata* and two *M. balbisiana* genomes), as cooking bananas. Seedless cultivated bananas that are diploids (AA or AB) exist as well.

In 2017, global banana production was 114 megatons, with locally consumed bananas, often grown by smallholder producers, making up 80% of global production. Smallholder organic agrosystems rely on 4 to 22 banana varieties[4] mixed with other food crops and often with trees[5] (Fig. 1a). This is in striking contrast with commercial plantations, where large fields consisting of one single clone from the 'Cavendish' subgroup (*Musa* AAA; Fig. 1b) are managed with agrochemicals, even though intermediate systems are emerging[6]. The monoculture practice directly relies on a very narrow and inflexible genotypic background in the crop. Hence, *Foc* TR4 creates havoc in these monocultures with genetic uniformity[7]. However, in smallholder fields, backyard gardens or mixed agrosystems, the predecessor of 'Cavendish' called 'Gros Michel' (*Musa* AAA) and other varieties similarly susceptible to *Foc* race 1 (R1) are still used even today[4,8]. During the first part of the 20th century, 'Gros Michel' was the most internationally traded banana due to its favorable traits, i.e. big bunch, bruise-resistant peel and ability to withstand the long journey from farm to market[9]. However, the highly susceptible 'Gros Michel' variety was completely replaced by resistant 'Cavendish' bananas in the large-scale industrial plantations during the 1950-1960s, as Fusarium wilt caused by *Foc* R1 rapidly spread across South and Central America[10].

Fig. 1 Two contrasting banana cropping systems. a, Organic farm in Tanzania with 'Mchare' banana (AA genome group) intercropped with coffee and in the shade of big trees, ensuring a richer above- and belowground biodiversity. **b**, Conventional banana plantation in Honduras based on the monoculture of 'Cavendish' (AAA genome group). Credit: Rony Swennen.

Research priorities and complementary strategies to control Fusarium wilt of banana

Biodiverse agrosystems to increase resilience

Unlike other banana fungal diseases such as Sigatoka leaf diseases, which are largely controlled by fungicides with up to 50 sprays per year, Fusarium wilt cannot be controlled unless performing complete sterilization of the soil[11], which is unaffordable and moreover destroys the soil microbiome. Therefore, an effective and efficient mitigation management of Fusarium wilt should consist of a combination of strategies including redesigned banana cropping systems conducive to higher above- and belowground biological diversity.

Smallholder farms tend to rely on more heterogenous agrosystems with minimal inputs[5]. Noticeably, 'Sukari Ndizi', a local banana variety in Eastern Africa susceptible to *Foc* R1, can be cultivated under such heterogenous agrosystems in *Foc* R1 endemic regions[12]. Efforts are being made to identify specific bacterial and fungal genera present in asymptomatic 'Sukari Ndizi' plants and *Foc* suppressive soils, as they were demonstrated to host a wide diversity of microorganisms[12]. Cover crops in industrial banana plantations are a first good attempt to not only reduce chemical weed control, but also to reduce weevil and nematode infestations[13,14]. However, more research is needed to identify the ideal cover crops contributing to pest regulation and biomass production. Such cover crops should be able to grow in the shade of the banana plants and not compete with them. Banana varietal mixtures are an additional option, as practices in East and Central Africa showed that banana production with *Foc* R1 susceptible varieties is possible where *Foc* R1 is paramount[15]. It is in

such varietal mixtures where the *Foc* R1-susceptible 'Gros Michel' has not disappeared from biodiverse smallholders fields in Africa - nearly 70 years after the *Foc* R1 epidemics annihilated 'Gros Michel' plantations in Latin America. Moreover, such susceptible varieties are cultivated as part of intercropped or agroforestry systems in association with small trees like coffee, but also in the shade of big trees[16,17] (Fig. 1a). Agricultural management practices with increased level of biodiversity on the farm were shown to reduce the intensity of important fungal diseases in crops[18], including Fusarium wilt[19]. The mechanisms involved in these biodiverse agrosystems remain elusive. It is possible that higher biodiversity in the field triggers, directly or indirectly, the induction of resistance mechanisms in neighbouring plants through competition for resources (e.g. light, water, nutrients), the release of specific plant-derived compounds, or the establishment of plant-microbiome interactions[20].

In such biodiverse-rich environments, plants are exposed to different types of microbiota leading to complex plant - microbiome interactions, with considerable potential to increase plant health[21]. Indeed, the molecular signals that trigger plant immune responses are highly similar and often identical in pathogenic and beneficial microbes[22]. However, the beneficial effects of plant-associated microbiome are usually variety- and species-specific, and reveal robust habitat and genotype-dependent selections[23]. Therefore, functional plant - microbiome interactions should be incorporated into breeding processes as a trait for selection[24]. Nevertheless, further efforts need to be made in order to identify key genotype-microorganism interactions and candidate genes for *Foc* tolerance. To achieve this, a better characterization of the microbiomes in relation to banana genotypes, agricultural practices and environments would result in essential information to adapt banana breeding. For instance, microbiome profiles from tolerant and susceptible banana plants would help identifying those microorganisms and, ultimately, candidate genes associated wit *Foc* tolerance and/or inducing resistance. Likewise, identifying *Foc*-resistant accessions through germplasm screening would help to understand mechanisms of resistance and provide banana breeders with the genetic resources to be integrated into commercial varieties. Therefore, selection of naturally resistant varieties needs to tap into the available banana diversity[25]. Because soil microbiome impacts plant health, these new varieties should then be integrated into agrosystems and crop management practices that stimulate soil biodiversity associated with resistance against Fusarium wilt[19].

Precision breeding approach

With the export industry still highly dependent on its preferred ‘Cavendish’ varieties, it is also necessary to develop *Foc* TR4 resistant ‘Cavendish’ or ‘Cavendish’-like bananas. However, conventional breeding is time- and labour-consuming, especially in crops with long life cycles which require large plantation areas, such as banana[26]. In addition, although sources of resistance to *Foc* TR4 were found in wild banana species[27], introgression of *Foc* TR4 resistance genes into commercial varieties by conventional breeding remains a difficult task due to the sterile nature of ‘Cavendish’[26]. On the other hand, mutation induction resulted in ‘Cavendish’ and other varieties with only intermediate resistance[28]. Genetic transformation of banana offers the opportunity to overcome the difficulties of classical breeding[26]. Transformation of ‘Cavendish’ with *resistance gene analog 2* (*RGA2*), isolated from a TR4-resistant diploid banana, showed promising results[29]. However, acceptance of transgenic products by consumers, particularly in the European Union, prevents adoption of transgenic technologies by the banana export industry[30]. Therefore, new approaches to develop ‘Cavendish’ varieties displaying stable and complete resistance are urgently needed.

New Plant Breeding Techniques (NPBTs) are opening venues to breed difficult crops such as banana and can accelerate the transition towards precision breeding for crop improvement[31]. Polyploidy in *Musa* varieties is associated with domestication, and speed breeding techniques could be instrumental to rapidly reproduce domestication events and provide access to novel traits, including disease resistance, for subsequent selection of improved varieties[32]. Precision breeding using CRISPR technology also holds tremendous opportunities for rapid and direct editing of current elite triploid varieties. Genome editing of banana has been established using *Agrobacterium*-mediated stable genetic integration of a *Cas9*-containing transgene in the genome of sterile triploid varieties[33-36] (Fig. 2). *Agrobacterium*-mediated stable transformation offers the advantage of a high efficiency. However, the main drawbacks are the impossibility of out-crossing the T-DNA in triploid genotypes and the needed efforts to select well-characterized single-insertion events (Supplementary Figure 1). Proof-of-concept and optimization of genome editing methods in banana have largely relied on the inactivation of the *Phytoene desaturase* (*PDS*) gene in triploid genotypes as shown by ourselves (Fig. 2c) and others[33,35,36]. However, the drastic albino phenotype of *PDS* knock-out in banana significantly impedes plant regeneration and growth, thus hampering optimization of gene editing methods. By contrast, the gene coding for a subunit of the chloroplast signal recognition particle (cpSRP) machinery, *cpSRP43/CHAOS*[37] appears as a suitable alternative target to monitor genome editing efficiency and further advance genome editing protocols.

Indeed, CRISPR-Cas9 mediated knockout of *CHAOS* in banana leads to pale-green regenerants with normal *in vitro* growth (Fig. 2c).

Because stable transformation of sterile banana triploids does not allow subsequent transgene removal by outcrossing like those in other edited crop species[38], gene editing could also be performed at the pre-breeding stage by editing improved diploid parents. Since the generation of totipotent embryogenic cell cultures essential for *Agrobacterium*-mediated transformation (Fig. 2a) is genotype-dependent, this approach might encounter limitations with diploids, which are often recalcitrant to embryogenic cell induction from meristems[39]. Initiating such embryogenic cell cultures from immature zygotic embryos excised from seeds would, thus, provide an alternative[40]. Another approach that remains to be tested in diploid banana is the haploid induction editing (HI-Edit) technology, which combines haploid induction with gene editing[41]. The main haploid inducer locus is known to encode MATRILINEAL (MTL) and CRISPR-Cas9 knockout of MTL has been used to make haploid inducers in rice and wheat[42].

The sterile nature of triploid commercial varieties and the reluctance to use transgenic banana make DNA-free CRISPR-Cas delivery methods indispendable for direct gene editing. Transient T-DNA delivery using *Agrobacterium*[43] or the use of carriers such as nanoparticles[44] can help establishing T-DNA-free edited banana. Because CRISPR-Cas9 functions as a ribonucleoprotein complex (RNP), it can also be delivered as *in vitro* synthesized RNPs by biolistics to plant cells, as pioneered in maize and wheat[45,46], or to protoplasts[47]. Protocols for biolistics of cells and protoplast electroporation followed by plantlet regeneration were explored for banana in the past[48,49]. Biolistics or particle bombardment of banana cells is a relatively simple delivery system that remains constrained by low transformation efficiency and the production of chimeric plants when no selection marker is used (Supplementary Figure 1). Transformation systems of banana protoplasts ensure regeneration of non-chimeric plants but they are limited by the low viability of protoplasts after electroporation (Supplementary Figure 1). These aforementioned limitations, as well as the availability of good quality embryogenic cells cultures with low probability of somaclonal variation[50], will need to be addressed in order to establish routine DNA-free genome editing protocols for banana. Reducing tissue culture time by direct somatic embryogenesis using morphogenic regulators and by cryopreservation could limit somaclonal variation and make banana gene editing more efficient[51,52]. Cryopreservation would be executed as soon as enough quantity of good quality embryogenic cells suspensions is

obtained, leading to a long term genetically stable stock of totipotent cells. Additionally, optimizing the photoperiod and the light quality/intensity required for plant regeneration after transformation could be applied to shorten the process[53].

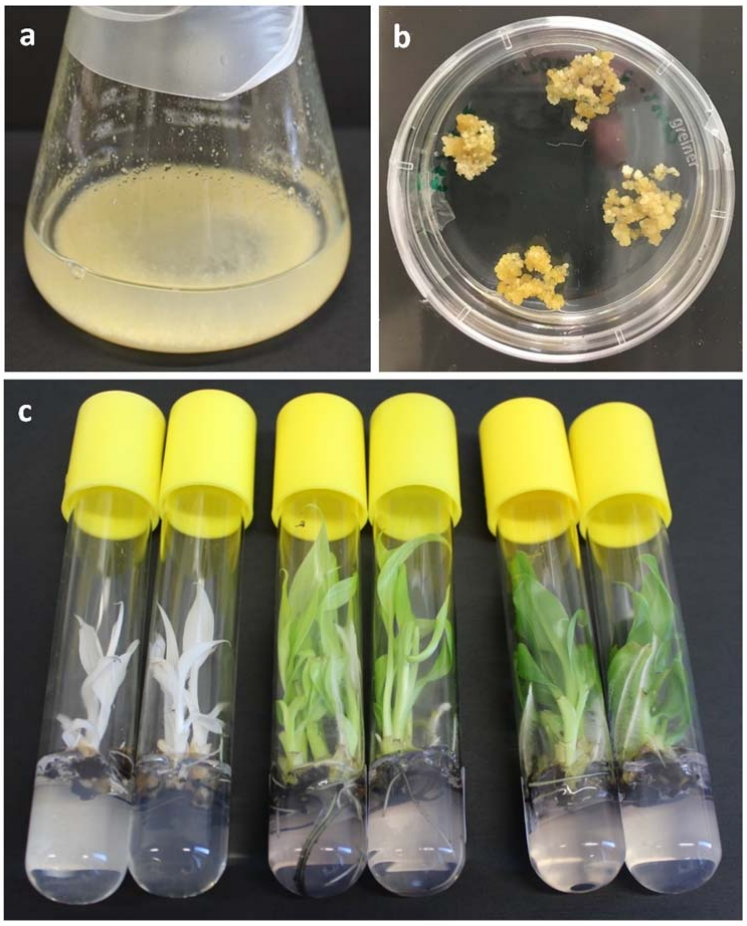

Fig. 2 Targeted genome editing of banana using CRISPR-Cas9. a, Embryogenic cell culture of 'Williams' ('Cavendish', AAA genome group) used for *Agrobacterium*-mediated transformation and genome editing. **b**, Transformed embryogenic cells growing on selective regeneration medium. **c**, Genome-edited plants showing: i) mutations in *Phytoene desaturase* (*MaPDS*) leading to albino phenotypes (left), ii) mutations in *Chlorophyll a/b binding protein harvesting–organelle specific* (*MaCHAOS*) leading to pale-green phenotype (middle), and iii) wild type phenotype (right). *Ma: M. acuminata*. Credit: Yasmín Zorrilla-Fontanesi.

Progress in NPBTs for banana needs to be concomitant with the identification of candidate genes for disease resistance. Despite several years of research, identification and characterization of genes conferring resistance against *Foc* TR4, the closely related subtropical race 4 (STR4) and R1 remain scarce[29,54]. Recent efforts to characterize the transcriptome modulation after *Foc* TR4 inoculation have led to the identification of a number of candidate resistance genes whose validation could be accelerated by fast and

robust NPBTs[55-57]. Additionally, the characterization of resistance genes in other *Fusarium oxysporum* – host plant pathosystems combined with genomics approaches might also narrow down natural resistance gene candidates in banana[58,59]. Strong candidates for genome editing in banana could be either negative regulators of disease resistance genes or host susceptibility genes, which have been used to generate loss-of-function mutations (knock-outs) in other plant-fungal pathosystems[31]. However, in perennial plant species that underwent recent whole-genome duplications, such as banana[60], a large proportion of genes belong to well-conserved gene families comprised of several paralogs with highly similar DNA sequence. Simultaneous expression of multiple single guide RNAs targeting different paralogs would allow to perform 'multiplex genome editing' (e.g., double, triple, quadruple mutants), as demonstrated in many plants, including rice[31], and provide a powerful tool for addressing the problem of genetic redundancy in banana. Likewise, the generation of gain-of-function mutants (knock-ins) of resistance genes by homology-driven repair is another option, although this method still remains difficult to implement efficiently in higher plants[31]. Alternatively, enhancing the expression of resistance genes in 'Cavendish'(-like) bananas, as the *RGA*[29] gene, by means of CRISPR-mediated gene regulation, targeted promoter mutagenesis or replacement[31,38] may also lead to the generation of transgene-free banana varieties resistant to *Foc* TR4.

The way forward

Current challenges in banana production will require a holistic approach building on new agronomic practices supporting biodiversity and the development of banana varieties requiring lower agricultural inputs (Fig. 3). Sustainable and immediate mitigation strategies for the *Foc* TR4 should rely on a combination of "smart" agrosystems[61] and cohort-based crop management practices[62]. Cohort-based banana management will also require a global surveillance system of pathogens to match banana cropping systems and risk management[63]. Due to the limitations inherent to banana genetics, breeding disease resistant bananas, including the 'Cavendish' dessert banana, represents a middle to long-term strategy. However, the potential of banana improvement to increase the durability of banana cropping systems cannot be underestimated. Concomitantly, implementation of such durable cropping systems will also ensure that the newly developed resistant varieties will hold longer in the field by slowing down the emergence of pathogens able to overcome the deployed resistance. High-throughput sequencing technologies have helped identifying soil microbiomes associated with plant health[21], and banana breeding programs could take advantage of such

approaches to develop resistance to Fusarium wilt (Fig. 3). Because the diversity of cultivated banana has long been impeded by its genetic structure, breeding programs also need to take advantage of recent progress in tools for genetic improvement to rapidly assess and introduce disease resistance genes in susceptible banana varieties (Fig. 3).

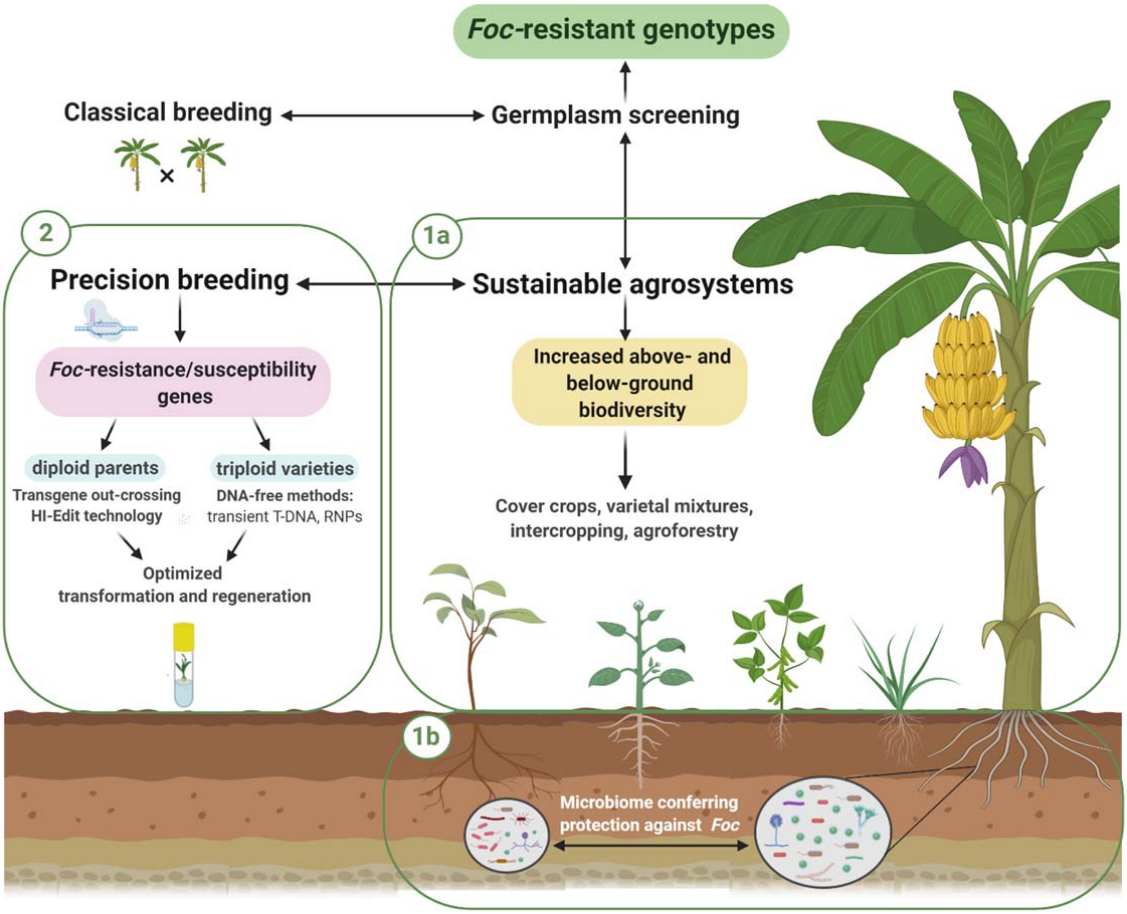

Fig. 3 Intergrated view of the proposed strategies for Fusarium wilt mitigation management in banana. Sustainable agrosystems increasing the above- and below-ground biodiversity on the farm (1a) can lead to the establishment of novel plant - microbiome interactions (1b) and the discovery of candidate genes associated to *Foc* resistance or tolerance. Concomitantly, New Plant Breeding Techniques (NPBTs), such as CRISPR genome editing, can be used for precision breeding in banana (2) and the generation of improved *Foc*-resistant lines through targeted modification of susceptibility/resistance genes either in diploid parents (pre-breeding stage) or triploid varieties. *Foc*: *Fusarium oxysporum* f. sp. *cubense*. HI-Edit : haploid induction editing technology ; RNPs : ribonucleoproteins.

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Author contributions

R.S., H.V. and Y.Z-F. led the writing of the paper. L.P., B.P. and S.S. contributed to the critical reading of the manuscript, provided suggestions and contributed to the writing of specific sections. Y.Z-F. composed Figures 1, 2 and 3. S.S. composed the Supplementary Figure. R.S. and H.V. initiated and coordinated the manuscript.

Competing interests

The authors declare no competing interests.