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Securing the future of Cavendish through precision gene editing

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ABSTRACT

Tropic is committed to revolutionize the future of Cavendish by combating the two existential threats of the banana industry (Panama Disease - TR4 and Black Sigatoka - BSD) and by significantly reducing yield losses. Tropic, founded in the UK in 2016, and comprised of over 150 business and technology professionals, is using cutting-edge technologies to produce improved gene-edited non-GMO elite Cavendish varieties. After the successful development of the first reduced-browning and extended-shelf-life varieties in 2025, we are advancing the development of our disease resistant varieties. From our large screen trials we have successfully identified the target genes responsible for conferring resistance to Panama disease (TR4) and Black Sigatoka (BSD) and we have developed non-transgenic lines that show promising resistance in field trials.

Keywords: Cavendish, TR4, BSD, Genome-editing

RESUMEN

Tropic está comprometida con revolucionar el futuro del banano Cavendish enfrentando las dos amenazas existenciales de la industria bananera: *Fusarium oxysporum* f. sp. *cubense* raza 4 tropical (TR4) y la Sigatoka Negra (SN), y reduciendo significativamente las pérdidas de rendimiento. Fundada en el Reino Unido en 2016, Tropic cuenta con más de 150 profesionales en negocios y tecnología que emplean herramientas de vanguardia para desarrollar variedades élite de Cavendish editadas genéticamente (no transgénicas). Tras el desarrollo exitoso, en 2025, de las primeras líneas con menor pardeamiento y mayor vida útil, la empresa avanza en variedades resistentes a TR4 y SN. Los ensayos a gran escala han identificado genes diana responsables de la resistencia y líneas no transgénicas con resultados prometedores en campo.

Palabras clave: Cavendish, Resistencia, TR4, Sigatoka Negra, Edición del genoma

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INTRODUCTION

Banana is the most traded tropical and subtropical fruit with 19 million tonnes of global export volume in 2022 (FAO, 2023) and the fourth most important staple crop. Global banana exports declined from 22.5 million tonnes in 2019 to 19.1 million tonnes in 2022 (FAO, 2023). Increased production costs of banana is one contributing factor to this trend which is partially driven by the increasing cost for disease prevention measurements (FAO, 2023). About 10-25% of banana production cost is spent to control black sigatoka, or black leaf streak disease (BSD) (*Mycosphaerella fijiensis*), which can lead to 50% yield losses if untreated (Noar *et al*., 2022). The main control for BSD is the application of fungicides which can be required at a rate of up to 70 applications per year and decreasing sensitivity to the commonly applied fungicides is an increasing concern (Noar *et al.,* 2022).

In addition, the presence and spread of tropical race 4 (TR4, *fusarium oxysporum* f. sp. *cubense*) further puts financial pressure on the banana industry (FAO, 2023). Even though strict control measures are in place, the recent reports of TR4 emergence in Colombia in 2019 (Garcia-Bastidas *et al.,* 2019), Peru in 2022 (Acuna *et al*., 2022) and Venezuela in 2023 (Herrera *et al*., 2023) show that these measures only slow down the spread, rather than prevent it.

Moreover, in the industry, it is estimated that between 10 and 15% of production loss is seen through premature ripening in the business chain. Premature ripening of fruit is also observed in plants generally infected with Black Sigatoka disease. The opportunity of extending shelf-life by delaying or slowing ripening offers a myriad of opportunities to growers and the entire business chain. Currently no known breeders are working on extended shelf-life products for bananas.

The triploid nature and sterility of Cavendish makes breeding attempts highly inefficient and the propagation of Cavendish is limited to *in vitro* culture.

At Tropic (Norwich UK), we apply genome editing (GE) using the CRISPR/CAS technology in a non-GMO manner to develop bananas with improved traits and the first lines with reduced browning and extended shelf-life developed by Tropic are now exempt from GMO regulations in Honduras, the Philippines, Costa Rica and US.

Here we summarise the findings of our disease resistance screening trials and provide an overview on our extended shelf-life variety and the development of new varieties resistant to TR4 and BSD.

MATERIAL AND METHODS

Transformation of Grand Naine embryogenic cell suspension and plant regeneration

To generate genome edited plants, plasmids containing Cas9 and sgRNAs (guide RNA) were designed to edit endogenous banana sequences. Banana ECS cultures were generated from immature male flowers (Cote *et al*., 1996) and transformed by cocultivation with *Agrobacterium tumefaciens* (Khanna *et al*., 2004). Plantlets were regenerated and propagated as described by Tripati *et al*. (2012). Two to four months after collection, the shoots were sampled for DNA analysis. Clonal propagation was initiated by exposing the meristem to propagation medium. Plants were refreshed on new media every 4 weeks until pathology and field trials were initiated.

Genotyping of regenerated events

Individual plant lines representing independent editing events were analysed for presence or absence of Cas9 using qPCR with specific primers and for the presence of the edits in the respective target gene or genes by sanger-sequencing. Only edited plants were analysed in pathology assays and field trials.

TR4 pathology assay

TR4 pathology assays were carried out as described by García-Bastidas *et al*. (2019). In brief: *in vitro* plants were grown in soil for 7 weeks and inoculated with 200 ml of 1E6 conidia/ml on 8-6-2023, monitored weekly and scored 6-10 weeks after inoculation depending on disease progression. Disease severity was determined by image analysis with ImageJ (ImageJ, vs. 1.52). Data was analysed in GenStat v23 using linear mixed modelling of repeated measures.

BSD field trials

Genome edited Grand Naine plants were grown for 13 weeks in the nursery and moved to field trials alongside industry standard Cavendish (BSD susceptible) and FHIA-25 (BSD resistant) plants in a randomized block design. In each trial a minimum of six clones of each edited line was tested with a single clone positioned randomly within each block at a density of 4444 plants per hectare. No fungicide treatments were applied for the duration of the trials. Trials were planted throughout the year. Disease pressure was assessed in each trial against BSD development on the susceptible Cavendish control variety. Four weeks after planting, different parameters were scored for 10 weeks: total leaves on the plant, oldest leaf clear of streaks (OLCS), oldest leaf clear of burns covering <5% (OLCB<5%) leaf area, oldest leaf clear of burns covering >5% (OLCB>5%) leaf area. In the cases of slower disease development on the

Cavendish controls in any trial during drier seasons, BSD evaluations were extended for additional scoring weeks. Data was analysed in GenStat v23 using linear mixed modelling of repeated measures.

Green life assay

Plants genome edited to extend banana green life were prepared in the nursery or field transplanting as described for the BSD trials. Approximately 100 clones of the genome-edited lines were planted in plots of three plants across five randomized blocks at a density of 1550 plants per hectare. Each block also contained clones of an industry standard Cavendish variety. The trial was managed following standard commercial practices for disease control and plant husbandry. Fruit was harvested after 91 days hanging. Harvested clusters are treated with a commercial solution of Thiabendazole-Alum and packed into boxes as commercially practiced, prior to storage in a cold room at 71.6°F (22°C). Green life was manually assessed twice a day (morning and afternoon) for the presence of soft pulp. The presence of soft pulp was considered the end of green life and the number of days taken to reach this stage after entering the cold room noted. The analysis of green life was carried out on one hand per bunch for at least 15 bunches per plant line. Data was analysed in GenStat v23 using a general linear model.

RESULTS AND DISCUSSION

Over 50 *Musa acuminata* genes showing relevance for resistance to TR4 and BSD were edited and approx. 10,000 plants were screened in field trials and greenhouse. From these, we have identified 6 target genes that, when edited, confer disease resistance in greenhouse and/or field. We will summarise here the results of the screening and the disease performance of the selected lines in the field and in greenhouse trials. We will also demonstrate that our non-transgenic edited lines display strong resistance in bioassays.

TR4 resistance screen of genome edited Grand Naine plants

Over 600 independent experimental edited lines (total 502 individual plants) covering 31 different target genes were screened in the greenhouse for TR4 resistance. From these we were able to select 3 target genes that consistently correlated with strong TR4 resistance in greenhouse and field. Figure 1 shows the disease score in greenhouse bioassays of the lines edited in the three genes (Gene A, Gene B and Gene C) compared with the susceptible and resistant controls. The disease score is measured in % corm area showing typical discoloration caused by TR4 infection. Lines edited in the 3 target genes display TR4 resistance comparable to that of the resistant FHIA control. Trials

conducted in non-TR4 infested field demonstrated that disruption in any of the 3 genes does not negatively impact agronomic performance. In addition, we have generated several non-transgenic lines edited in the 3 genes of interest which showed significant resistance against the fungus in greenhouse under high inoculum pressure (Figure 2). These lines will be tested in field trials in different geographies throughout 2026.

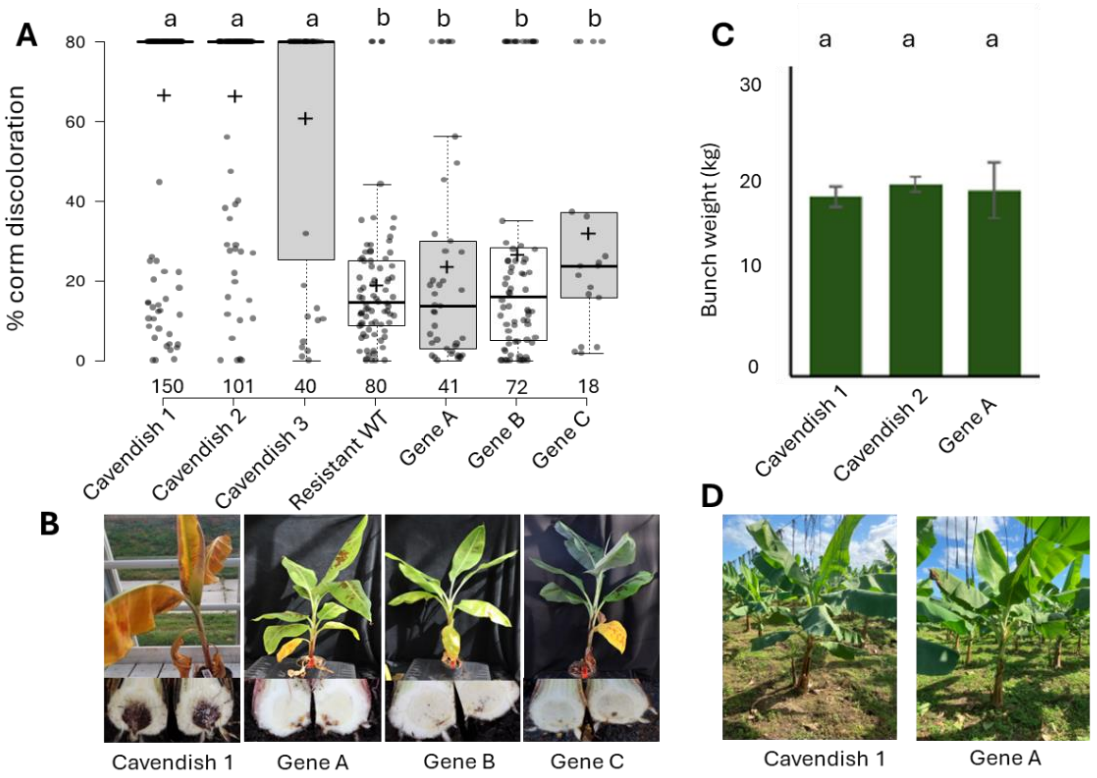


Figure 1. TR4 disease resistance and agronomic performance of the lines edited in the 3 top target genes. A: Assessment of corm discoloration in edited and control lines 6 weeks post-inoculation. The number under each graph indicate the number of plants scored. Lines edited in gene A, B and C show significantly increased resistance ($p \leq 0.01$) compared to all susceptible cavendish lines. B: External and internal TR4 symptoms. C: Example of agronomic performance (measured as bunch weight in kg) associated with disruption of Gene A. D: Plants edited in the 3 genes are indistinguishable from conventional Cavendish in the field. A representative picture of plants edited in Gene A vs Industry standard Cavendish is shown here.

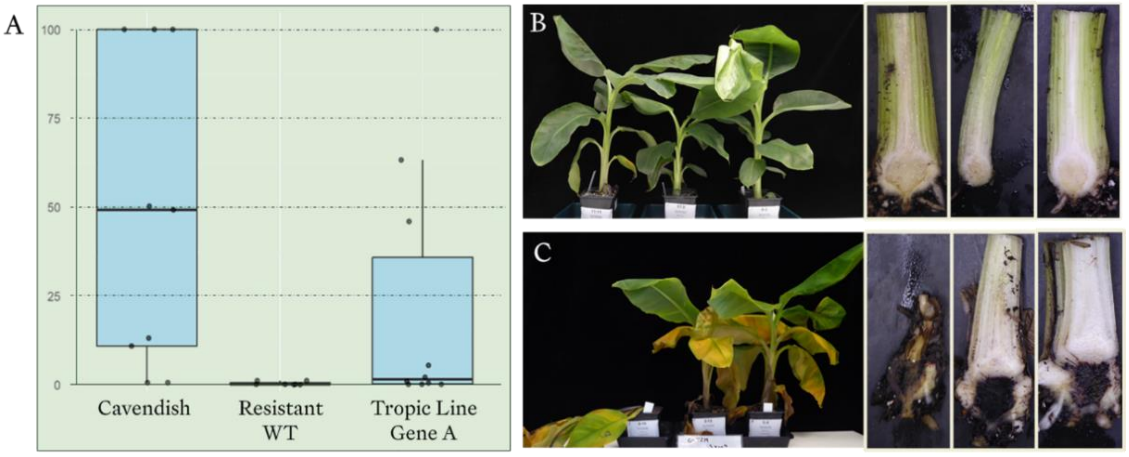


Figure 2. TR4 disease resistance in one of the edited non-transgenic lines. A: Assessment of corm discoloration in Tropic line and control lines 6 weeks post-inoculation. B: Tropic edited non-transgenic plants show no external or internal symptoms while Grand Naine controls (C) display visible symptoms of infection.

BSD resistance screen of genome edited Grand Naine plants

To identify Cavendish target genes able to confer resistance to BSD, approximately 800 independent edited lines covering 29 different target genes, individually and in combination, were screened in the field under different management regimes for BSD resistance. Each line represents a different editing event in a specific target gene or combination of target genes. Scoring was initiated 4 weeks after moving the plants to the fields and plants were typically scored for 10-11 weeks. The screening identified 13 independent lines (edited in 3 individual target genes) that showed significantly less BSD symptoms compared to the susceptible control with an average of 9 and 10 leaves clear of burns <5%. This value was comparable with the resistant FHIA-25 line (Example of resistance observed in lines edited in one of the three gene is displayed in Figure 3). The 3 top target genes have also been edited in combination and we have generated multiple non-transgenic lines from which we expect BSD resistance field data in early 2026.

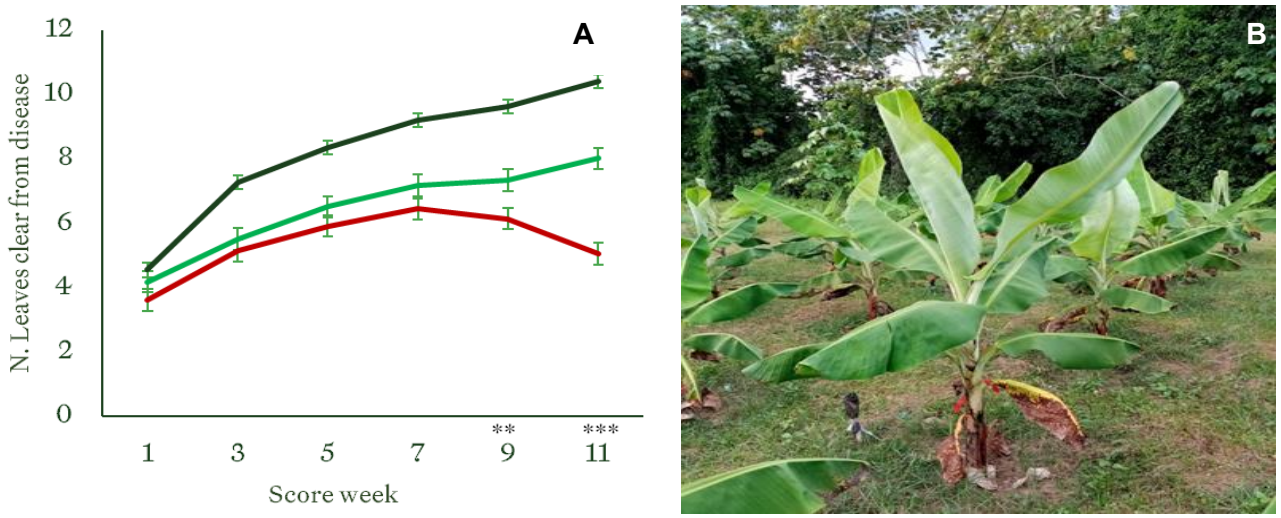


Figure 3. BSD scoring data of top performing edited lines in BSD field trials 7 weeks after planting. **A.** Plants edited in one of the top target genes (light green) show significantly reduced BSD symptoms compared to Cavendish industry standard control (red) and are comparable to the resistant FHIA-25 line (dark green). * indicates significant difference compared to Cavendish control. **B.** Editing of the target genes does not affect agronomic performance and plants are indistinguishable from Cavendish industry standard.

Tropic Extended Green-life Variety

To extend the green life of banana fruit and reduce losses due to premature ripening, we have edited genes involved in the ethylene biosynthetic pathway. Our new edited non-transgenic variety (Aura) displays an extension of green-life of up to 12 days (Fig. 4A) and Cavendish commercial agronomic performance (Fig. 4B-C).

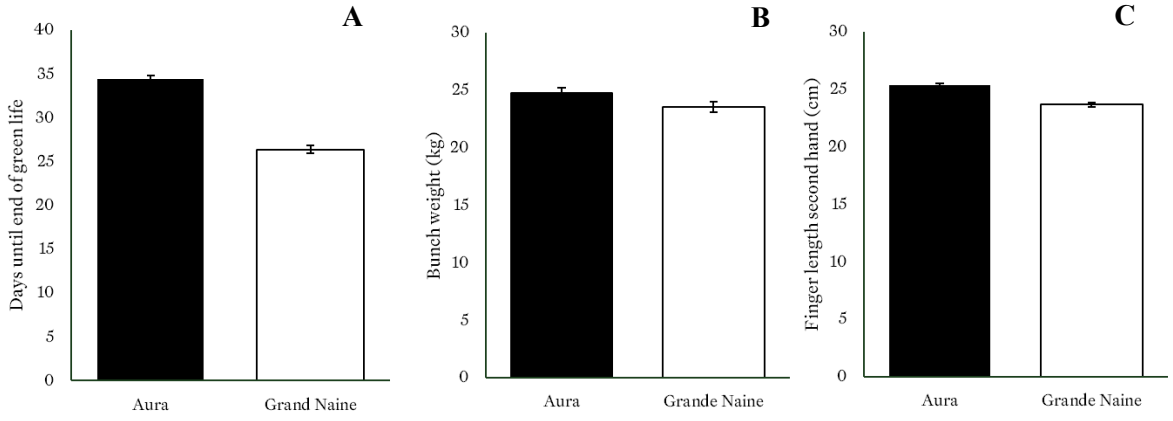


Figure 4. Performance of our new variety Aura vs conventional Cavendish Grande Naine. A: Days until the end of the green life. B: Bunch weight. C: Finger length.

CONCLUSIONS

We have identified the genes responsible for conferring resistance to TR4 and BSD and we have developed multiple non-transgenic plant lines edited in those genes, which show resistance in greenhouse bioassays. We have also developed a new Cavendish variety with over 10 days extended green life.

Tropic's novel Cavendish varieties will effectively address the needs of the banana industry today and secure the future of the most loved fruit globally.

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