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"Mysterious Disease" of Maize: A Diagnostic Puzzle with a 20-Year History

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Diagnosing plant diseases is often complex and uncertain, particularly when routine tests fail to identify a clear cause. This case study highlights the intricate process of plant disease diagnosis by investigating a mysterious maize disease that took over 20 years to resolve. The study initially involved routine diagnostic procedures, including the isolation of fungi and bacteria from affected maize samples, as well as virus detection. Despite exhaustive efforts, no pathogens were identified as the cause of the observed condition. After two decades, advancements in genetic engineering and additional literature review led to the hypothesis that the issue might be related to maize genetics rather than an infectious disease. The investigation revealed that the maize variety in question had a genetic predisposition to excessive tillering, a trait associated with the *teosinte branched1* (*tb1*) gene. This trait, likely introduced as a by-product of genetic engineering efforts to improve maize

resistance to pests, could have caused nutrient depletion and poor crop performance under conditions with limited resources, rather than to a pathogen-induced disease. This case underscores the importance of integrating knowledge about molecular genetics and comparative genomics in plant disease diagnostics. It is recommended that diagnosticians consider interactions between genetic factors and environmental conditions in their assessments, moving beyond traditional pathogen-centric approaches to improve diagnostic accuracy and crop management strategies.

Introduction

Routine plant disease diagnostics involve a complex and intense workflow. A diagnostician receives a new case, performs the necessary procedures to identify the source(s) of problems, reports results, and moves on to diagnose the next case in the queue. Often a diagnostic task can be formulated as straightforward as conducting a specific test to confirm the presence of a given pathogen in a plant population. However, this perceived simplicity hides the existing level of uncertainty and inherited complexity of any diagnostic process (details can be found in Lenskaia et al. (2023)). For example, the threshold for reporting disease versus no disease for a diagnostic test can be arbitrary, with many factors that contribute to the degree of certainty in the diagnostic results.

The relationship between a diagnostician and each case is supposed to be neutral. This seems to be ideal for an unbiased investigation. The high psychological pressure associated with a possible incorrect diagnosis and moral responsibility for subsequent losses can stall any diagnostic process. Fortunately, the consequences of a reported diagnosis (or lack of it) are often very distant and somewhat obscure for a diagnostician. For example, if the sample appears to be positive for a certain plant disease, then a diagnostician rarely recognizes the complete damage it will cause for the farmer or plant grower to eliminate the affected plants and manage the subsequent physical and financial losses. The consequences can be as wide as the rollback and disposal of all of the thousands of affected plants sold to numerous customers around the world and as deep as the collapse of a farm business or even an entire industry. Thus, the total cost of a diagnostic error can be quite high.

The cost of an error is a very important factor that affects the quality of the diagnostic process and motivates further improvement of diagnostic procedures. Therefore, improvements in diagnostic technologies gain a lot more attention in medicine than in plant pathology. The international medical community has dedicated a lot of time, resources, and effort to the development of universal diagnostic algorithms and a unified international classification of diseases. In medicine, the final evaluation of the quality of medical diagnostics and subsequent treatment are done postmortem by an independent expert in pathology. Thus, doctors have extensive, yet expensive, opportunities to learn from diagnostic mistakes to prevent them in the future. Sadly, such learning experiences and opportunities for improvement are uncommon in massive plant disease diagnostics.

It is important to note that, in some cases, the diagnostic tests performed can return borderline results because of the limitations of their sensitivity and specificity. Sometimes it is a consequence of a cross-reaction when a positive result is a consequence of a "false shot" rather than a true call related to a suspected pathogen. In addition, workflows in the process of conducting tests can affect the overall quality of the results. All of these factors can significantly complicate and burden establishment of the correct diagnosis.

Moreover, complex diseases encompass a lot of unknowns that impact the progression of the disease and clinical outcomes for each individual patient. The development of comparative genomics and high-throughput sequencing technologies have paved the way for personalized medicine and novel treatment methods tailored to the genetics of individual patients. The dissemination of genome analysis methods has a significant impact on other areas as well. Some cases wait for many years to be solved, for the time when all of the necessary pieces of the diagnostic puzzle fall into place. This study is about a case that took us more than 20 years to unravel.

Cast of Characters

Jason Smith and Tracy Barlow, diagnosticians at the Plant Disease Diagnostic Laboratory

Sarah Brown, a commercial grain farmer

Case Objectives

The major goals of this case study are

1. To expose students, researchers, and plant growers to the main challenges related to diagnostics.
2. To encourage people who are involved in the process of disease diagnosis to think critically and look outside the box, taking into account a picture that is as broad and complete as possible when they try to identify the source of the problem.
3. To demonstrate a possible scenario using a real-life example when the diagnosed case extends way beyond the usual time allocated for a diagnostic process.

The Case

Part A

Samples of maize plants were sent to the plant virology lab by Sarah Brown, a seasoned commercial maize grower with a thriving operation. Sarah's family farm business had been the pride of their community for years. During this season, however, a shadow of uncertainty descended on her fields as Sarah had taken chances by planting a new maize variety recommended by a large buyer. She had hoped for a better yield and higher profits. As the days turned into weeks, however, Sarah could not ignore the worrying signs in the field. The leaves of the maize plants were wilting, and the once vibrant crop was now a sea of yellowing stalks. The implications were clear: if this new maize variety failed, it would mean more than just lost income for Sarah and her family. They had invested their time, energy, and resources into this crop, and their very livelihoods hung in the balance. Sarah could not afford to be a passive observer. She needed answers, and she needed them fast. What was the source of a mysterious disease that had taken root among her maize plants, affecting yields and threatening her livelihood? Sarah, known for her unwavering determination, found herself in a race against time and nature, seeking answers to protect her cherished crop and the generations of hard work that had gone into sustaining her family's business.

Sarah contacted the Plant Disease Diagnostic Lab and asked for help. She explained that the seeds of the affected variety were purchased from

SuperSeeds, a large seed-producing company. Sarah noticed that two maize varieties planted next to each other demonstrated very different responses to the very same care. Amid a warm, moderately humid summer, a mysterious disease had taken root among some of her maize plants. It struck not just in some areas but seemed to specifically affect plants of one particular variety, leaving her with substantially reduced yields. Despite Sarah's immediate efforts to rescue the crop using fungicides and meticulous irrigation, the elusive nature of the disease left her desperately searching for answers.

There were two maize varieties that grew in the field next to one another. One variety grew as expected and looked healthy. Another variety demonstrated stunted growth, bushy appearance, brown leaves, and wilting. Sarah sent samples of the affected plants to the Plant Disease Diagnostic Lab. She was desperate to know the source of the problem because the plants of the affected variety would barely produce kernels, leading to almost no yield.

Jason Smith, a diagnostician at the lab, examined the plants and plated samples on several different media. He isolated several species of bacteria and fungi from plant samples of the affected maize variety, and they were common saprophytes. The maize plants were colonized by a myriad of fungi, bacteria, and other microorganisms, but they seemed to be the lucky riders rather than the drivers of the observed poor plant conditions. Although bacterial colonies and fungi were flourishing by taking advantage of the weakened immunity of the maize plants, the origin of the observed unhealthy state was not clear since none of the species isolated were previously known to cause diseases. Jason contacted Sarah to ask questions and learn more details about the case. Sarah mentioned that there was no difference in care for the two maize varieties she planted next to each other in the field (e.g., soil, fertilizers, irrigation, and fungicide application). It seemed that no abiotic agent was to blame for affecting the plants. Could it be a novel virus?

Jason conducted viral detection procedures, including extraction, purification, and TEM screening (details can be found in Lenskaia et al. (2022)). None of these techniques revealed evidence of plant viruses. The source of the problem causing the observed set of symptoms was unclear. The disappointing results of the diagnostics were reported to Sarah who was perplexed. Consequently, the farmer regretted the experimental growing of the problematic variety and decided never to plant it again. Unfortunately, the diagnostic puzzle remained unresolved.

List of Possible Topics for Discussion

1. Occurrence of the disease
2. Disease spatial and temporal characteristics
3. Impacts of agricultural practices
4. Fungicide application practice
5. Impacts of environmental conditions
6. Source of affected crop varieties

Part B

Over the following years, Jason encountered other diagnostic cases related to maize diseases that were abundant. Some of them brought back memories of the unresolved puzzle of the "mysterious disease" of maize. That case kept coming to mind from time to time, with no apparent way to untangle the diagnostic riddle. Jason had not been able to find a feasible explanation and resolve the mystery of two maize varieties growing next to each other with such striking differences in their health and yields. Later, SuperSeeds discontinued the production and sales of the seeds for the variety that caused problems for Sarah's family business. Advances in crop genetic engineering led to preceding varieties being substituted for newly developed ones with better qualities. A few years later, Jason moved with his family to Chicago and started his own plant disease diagnostic lab.

Twenty years later, Tracy Barlow, the lab's lead diagnostician, was working on a diagnostic guide for maize diseases and searching through some old reports when she stumbled upon documents describing the unsolved maize disease case written by Jason Smith. She knew Jason from her previous interactions with him related to several difficult diagnostic cases that his lab helped to resolve. His expertise in exotic plant diseases was invaluable. Tracy decided to contact him about the mysterious case. Their comprehensive discussion of the case made them undertake another attempt to identify the source of the mysterious maize disease.

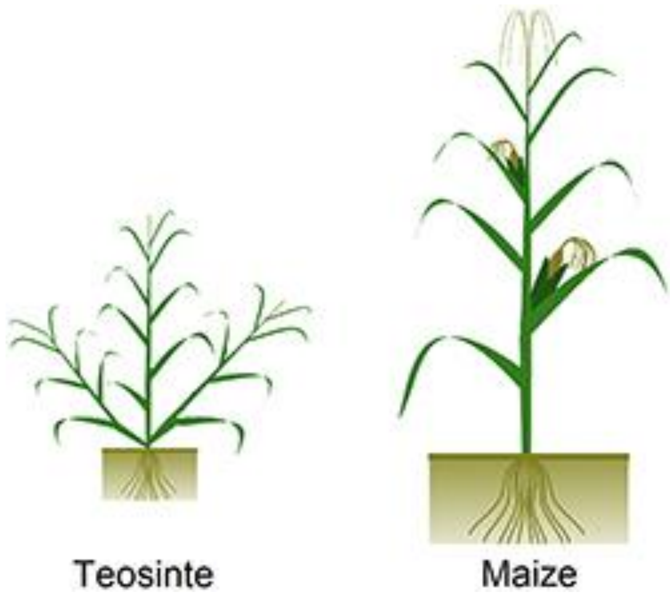

Fig. 1. Illustration of teosinte and maize plants.

Tracy and Jason did a comprehensive literature search and found several reports about field trials of the maize variety in question. The variety was called "Spark1235" and was developed using genetic engineering to improve resistance to pests. Tracy and Jason also found an interesting news article about a farmer who won a prestigious corn-yield competition using this particular variety. However, the winner was criticized for using several times more fertilizer on the contest plot than was recommended. The winning variety had a remarkable yield that was considered a great record. This gave Tracy and Jason an important clue to resolve the mysterious maize case. It seemed feasible to them that the poor condition of the maize might not be due to a "mysterious" pathogen for which Jason had so intensively searched. It might be an indicator

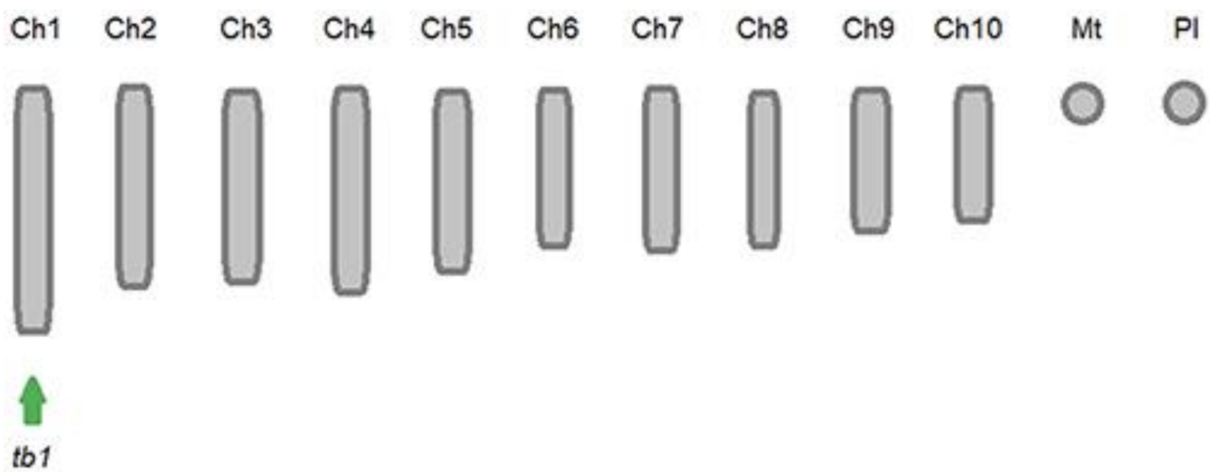

Fig. 2. Schematic representation of maize genome: 10 chromosomes, 1 mitochondrion, and 1 plastid.

of a completely different problem related to plant health.

The bushy appearance of the maize plants previously sent to the lab by the commercial grain farmer, Sarah, indicated that it was prone to forming excessive side branches (tillers). Tillers consume a lot of nutrients, and the plant can be exhausted if the amount of nutrients in the soil is limited. However, this property can be advantageous in terms of total yields if the plant has all the nutrients it needs in abundance. The likely wild ancestor of maize, teosinte (Fig. 1), has a bushy appearance (Doebley et al., 1997), and it distributes nutrients to all of its stalks. In contrast, maize usually concentrates resources in the main stalk, with suppression of side stalks.

The *teosinte branched1* (*tb1*) gene located on chromosome 1 in the maize genome (Fig. 2) is one of the key genes associated with the difference between teosinte and maize in apical dominance (Doebley et al., 1995) (i.e., the dominance of the central stalk over the side stalks). The product of this gene is a transcriptional factor, a protein that regulates the expression of other gene(s). The gene plays a crucial role in regulating branching patterns in plants. It was discovered in teosinte, a wild grass species that is a likely ancestor of modern maize (Yang et al., 2017), and mutations in this gene facilitated the process of domestication (Dong et al., 2019). This gene is part of the TCP family of transcription factors and is involved in the control of axillary bud growth.

The *tb1* homologs have been found in other plant species (Fig. 3). In Poaceae, *tb1* homologs are found in other grasses, including rice (*Oryza sativa*) and wheat (*Triticum aestivum*). Similar genes that influence branching patterns have been identified in tomato (*Solanum lycopersicum*) as well (Martín-Trillo et al., 2011). The presence of *tb1*-like genes in various plant lineages indicates that this gene family likely has ancient origins and has
been subject to subsequent changes and adaptations during plant evolution.

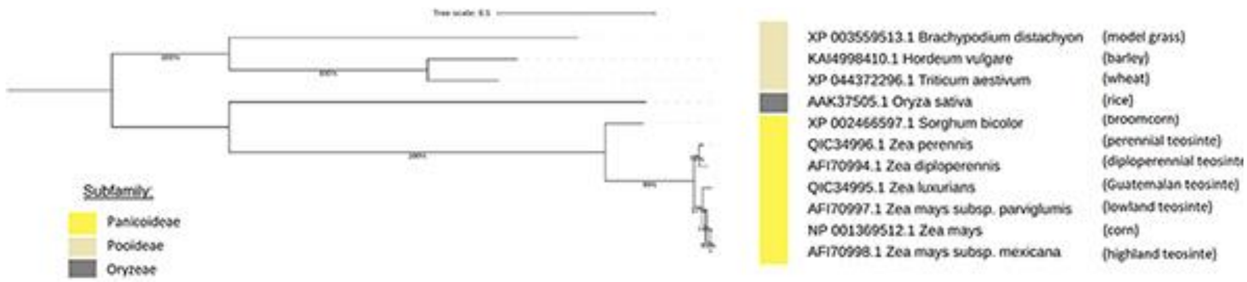

Fig. 3. Phylogenetic tree of teosinte branched1 (tb1) homologs. The tree is rooted at the midpoint.

The observed effect of excessive formation of tillers in the examined maize plants could be a side effect of changes in the maize genome introduced by genetic engineering during the development of this variety. In this case, the observed striking difference between two maize varieties planted by Sarah many years ago was likely not due to a disease. The difference might be due to the distinctly different nutrient demands of the two maize varieties resulting from the introduced genetic changes. It is worth noting that modern methods of genetic engineering for crops have progressed far beyond the methods developed for molecular genetic diagnostics in plant pathology. Twenty years ago, plant disease diagnostics did not have the capacity for comparative genome analysis necessary to solve the encountered diagnostic puzzle.

Today, the situation is changing. Researchers and diagnosticians consider knowledge in molecular genetics and plant genomics as pivotal assets in the realm of plant pathology, serving as a foundational tool for understanding the intricate relationships between plants and pathogens. By delving into the genetic makeup of both plants and the organisms that infect them, researchers gain invaluable insights into disease mechanisms, host-pathogen interactions, and plant defense strategies. Today, the knowledge harnessed through genomic techniques helps guide the development of disease-resistant crop varieties, informs sustainable agricultural practices, and enhances the ability to manage plant diseases effectively. The advances in molecular genetics and genomics are very important for the improvement of diagnostic methods in plant pathology, and they are key for safeguarding our global food supply and developing resilient agricultural systems in the face of evolving pathogens and environmental challenges. Their role in advancing our understanding of plant health and fostering food security is pivotal.

List of Possible Topics for Discussion

1. Role of literature review in disease diagnostics
2. Benefits and drawbacks of genetic engineering for crops
3. Genetic factors and apical dominance
4. Impact of molecular genetics on plant disease diagnostics

Classroom Management

Case Summary

This case study emphasizes the complexity of disease diagnostics and describes possible directions in the diagnostic process that can provide useful clues and significantly affect the quality of the diagnostic results. Stimulating students and researchers to think outside the box is the primary outcome of this educational experience.

Suggestions on How to Use This Case

This case study can be used to stimulate a class discussion (20–45 min) or fill in a laboratory session (60–120 min) on plant disease diagnostics for undergraduate and graduate students in plant pathology, horticulture, and other related areas. This case study aims to explore the routines of a plant disease diagnostic laboratory and to communicate important examples of diagnosed cases.

Background information about maize agriculture and genetic modification of crops can be assigned as a reading before the class follows up with a short quiz at the beginning of the class. Alternatively, the course instructor may allocate time at the beginning of the class session to review the materials in groups of three to four people by randomly assigning one of the suggested topics to each group (lists of possible topics for discussions are provided at the end of sections Part A and Part B). After the review, groups should formulate at least two key points about the assigned topic and share them with the class.

After the background information review is completed, the instructor will introduce Part A of the case study to the class by briefly describing the case settings and preliminary diagnostic steps that were taken. The goal of this step is to set the scene for a modeled conversation between the parties involved in analyzing the case. The instructor can write the list of suggested topics for questions on a whiteboard or distribute it as a handout. Students can be divided into groups of three or four people. Each group may pick an area from the list of suggested topics (provided at the end of section Part A) for questions and formulate two or three follow-up questions for a diagnostician to clarify the case and decide on the next step in disease diagnosis. Alternatively, this conversation can be role-played by dividing students into two groups representing a farmer and a diagnostician respectively. The questions can be discussed in class and addressed by the instructor in the context of the case. At the end of Part A, students should come up with a plan for possible next steps in the plant disease diagnostic process. The instructor will guide the discussion of the next steps to emphasize the possible sources of the observed conditions and ask about necessary steps to check the symptoms.

In Part B, the instructor will summarize the evidence collected so far from the preliminary diagnostic steps, the information received from all parties involved, and the viral detection results and formulate a diagnostic challenge. The instructor can stimulate a discussion with students related to other possible causes of the observed symptoms and insights about what might cause them before the class dives into the solution of the diagnostic challenge outlined in section Part B. As an option, the class session can be concluded by a class discussion. Alternatively, students can share their feedback and comments about the lessons learned during the case study in groups and then share the key points with the class.

Possible Adaptation

This case study can be adapted to accommodate specific education needs and available resources. Discussion can be enriched by demonstrating the materials related to plant disease diagnostics (e.g., plating of samples on different media and analyzing the results). This additional learning experience can be provided in collaboration with a plant disease diagnostic laboratory if time, resources, and interest allow. This case study emphasizes the interdisciplinary nature of disease diagnostics and its complexity. Stimulating students and researchers to think outside the box and deepen their understanding of the complexity of diagnostic challenges are the primary learning outcomes of this educational exercise.

Precase Quiz

1. What statement best describes plant disease diagnostics?

1. It is a well-established technology, and its success depends on how well a diagnostician can follow the existing protocols for diagnosis of any plant disease.
2. It is an art that depends on the creativity and diagnostic skills of a given person with no space for automation at any stage in a disease diagnostic process.
3. It is a science that requires addressing each new case as a research challenge and needs both creativity and technology to solve a diagnostic puzzle.

2. The results of each diagnostic case reveal the source of the disease.

1. True
2. False

3. What are possible sources of diagnostic mistakes?

1. Not following protocols for diagnostic tests
2. Lacking important details about the case
3. Overstating borderline test results
4. All of the above
5. None of the above, disease diagnostics is flawless

Answers to Precase quiz

1. c
2. b
3. d

Background Information

- Maize Growth and Development
- Nutrient Management
- Genetic Modification of Crops

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