Plant physiologist Hermann Prodjinoto takes molecular-level journeys into a familiar crop. Tomatoes are the most widely harvested fruits on the planet and are cultivated in nearly every country.

THIRSTY TOMATOES

By Dan Rubinstein
Photographs by Hadas Parush

Unravelling the relationship between water channels known as aquaporins and drought tolerance may be the key to feeding a drier and more crowded planet

Like all living things, plants need water. Rain falls from the sky and seeps into the ground, where root networks absorb the water. As it moves through the plant's anatomy, water helps distribute organic material and plays a critical role in photosynthesis. Powered by the sun's energy, photosynthesis maintains the oxygen levels essential for human and animal life and underpins the growth of fruits and vegetables.

While this system is remarkable, it is not entirely efficient. Less than 5 per cent of the water absorbed by plants contributes to cell expansion and growth, with the majority being released as water vapour, a process known as transpiration. When the stomata — tiny pores on the leaf's surface — open to allow carbon dioxide entry for photosynthesis, an estimated 400 water molecules are lost for every carbon dioxide molecule absorbed.

Transpiration, however, is vital to protect leaves from overheating and to ensure the continuous movement of water and nutrients from the roots to the leaves. Could improving water-use efficiency — by reducing excessive transpiration without compromising nutrient uptake — offer a pathway to developing hardier crops? Could this be a way, asks plant physiologist Hermann Prodjinoto, to help feed millions of people whose survival depends on crops that are susceptible to drought?

Prodjinoto, an Azrieli International Postdoctoral Fellow at the Hebrew University of Jerusalem (HUJI), thinks a lot about water — and tomatoes. The most widely harvested fruits on the planet, tomatoes are cultivated in nearly every country, including Prodjinoto's native Benin in West Africa. They are also an incredibly valuable agricultural product, with a global market worth more than $178 billion USD in 2023. And from a scientist's perspective, this member of the Solanaceae family is a model species to study because of its biological and genetic similarities to relatives such as peppers, eggplant and potatoes.

To enhance water-use efficiency in tomatoes, particularly under stress conditions such as drought and salinity, Prodjinoto is focusing on aquaporins. These proteins, often referred to as "water channels," regulate the movement of water molecules through cell membranes, playing a vital role in maintaining water balance. This "plumbing system" was only identified in 1992, a discovery that earned Peter Agre the 2003 Nobel Prize in Chemistry.

"While we have made significant progress in understanding the role of aquaporins in water transport and stress responses," says Prodjinoto, "there is still much to uncover about their precise molecular and physiological functions in plants. If we can learn more about the roles they play, we can use that knowledge to grow food more efficiently under challenging conditions. Aquaporins are a critical component in improving water-use efficiency."

Prodjinoto was born in Cotonou, the largest city in Benin, but moved to his mother's rural hometown as a child. One of his uncles was a farmer in the area, sparking his early interest in agriculture and plant science (see "Defying Drought"). Another uncle was a math teacher, and young Hermann showed an aptitude for mathematics, which would later complement his scientific pursuits. Inspired by his family and encouraged by his mother, he saw science as a way to address real-world challenges. "I wanted to focus on practical applications rather than purely theoretical work," says Prodjinoto. "I saw how science could help me make a tangible impact."

Prodjinoto discovered his passion for biotechnology and microbiology while studying at Benin's University of Abomey-Calavi. He saw the potential of these disciplines to improve agricultural productivity and address food security challenges. Building on this interest, Prodjinoto studied the impact of salinity on the root systems of rice during his PhD at Belgium's University of Louvain, where he discovered that aquaporins significantly influence the ability of rice plants to maintain yields in saline conditions.

Such insights about aquaporins will yield big dividends. By understanding how they work, how they are regulated during plant development and how the genes that code for these proteins are expressed, breeders will be able to develop more efficient plant varieties that are more resistant to water stress, says Stanley Lutts, who was Prodjinoto's PhD supervisor along with Christophe Gandonou.

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Lutts describes Prodjinoto as enthusiastic, dynamic and passionate, and as a scientist who promises to make advances in both fundamental and applied science. "Fundamental and applied research are not opposite or incompatible — they are complementary. The area of Hermann's research is an ideal illustration of this. It is impossible to progress without a strong foundation, and it is absolutely necessary to progress to feed humanity during the next decades."

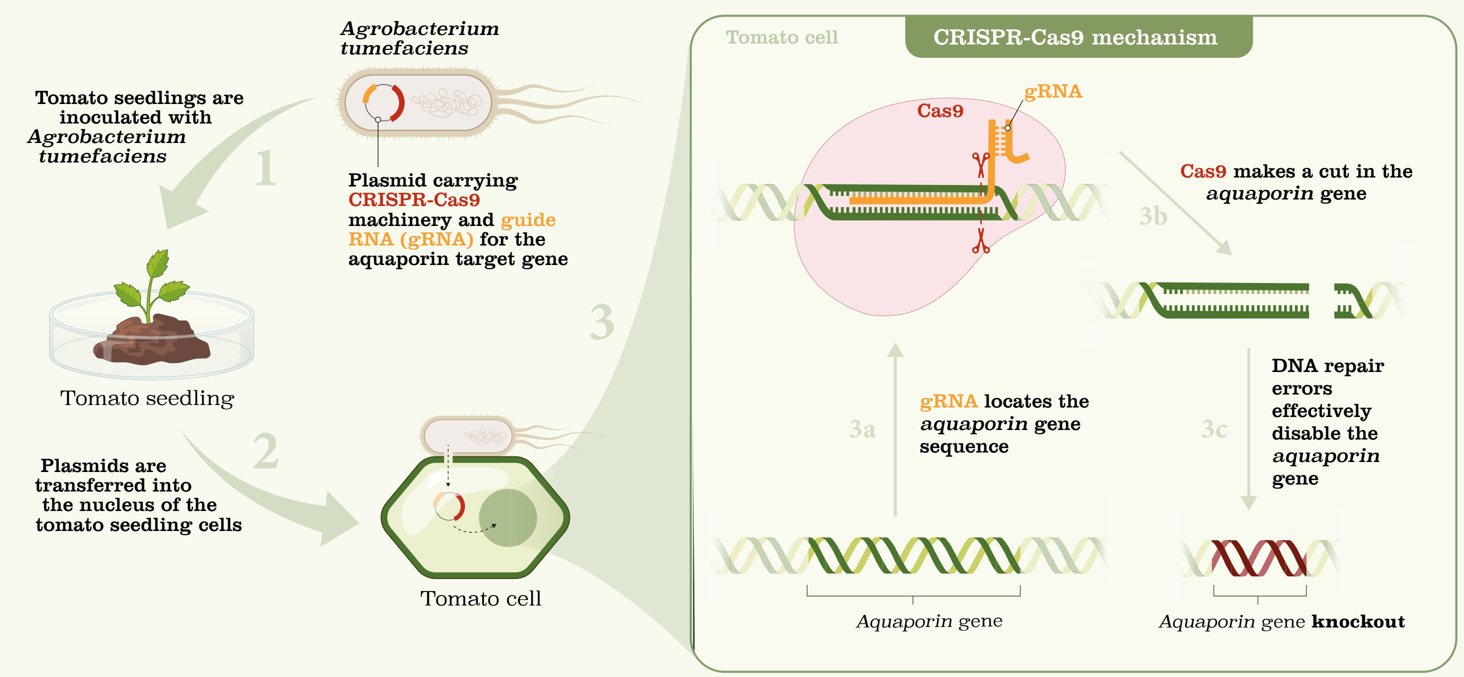
The stakes are high. Less than 1 per cent of the Earth's water is accessible freshwater, according to the United Nations, and roughly 70 per cent of it is consumed by agriculture. This supply is diminishing as warmer temperatures increase evaporation, glaciers recede and snowpack declines. With the global population projected to rise from just over eight billion today to 9.7 billion in 2050, food security is under threat.

Currently based in Rehovot, Israel, Prodjinoto conducts research at both the HUJI Faculty of Agriculture, Food and Environment and the Volcani Center, Israel's national agricultural research organization. "While I may not have the tools to reverse climate change, I can help plants adapt to its challenges," he says. "My research has the potential to reduce agriculture's reliance on freshwater resources, an especially critical need in arid and semi-arid regions. This project could equip farmers with robust tomato varieties capable of maintaining stable yields under suboptimal conditions."

Prodjinoto's postdoctoral project, conducted as part of his Azrieli Fellowship, is based on a straightforward concept — how aquaporins affect drought tolerance. Nevertheless, it is complex in methodology and scope. The tomato genome contains 47 aquaporin genes; by comparison, humans have 13. These genes are expressed across vegetative tissues (roots, leaves, stems) and reproductive tissues (flowers, seeds, developing fruits), suggesting their widespread involvement in plant survival and reproduction under stress. Prodjinoto is working to unravel the specific and combined roles of these aquaporins in the tomato's drought response.

He will be using gene-editing technology known as CRISPR-Cas9 to individually "knock out" the 47 aquaporin genes. Next, he will germinate and plant several hundred tomato seedlings — including wild and transgenic varieties — first in a greenhouse and then eventually in the field. He plans to monitor and compare their growth under conditions ranging from well-irrigated to severely water-limited. This research will generate detailed maps of the physiological functions and phenotypes associated with each aquaporin gene under the stress of drought and hopefully help lead to tomato varieties capable of thriving with limited irrigation.

Prodjinoto discovered his passion for biotechnology and microbiology while studying in his native Benin. Whenever he returns home, he speaks to students at local universities and collaborates with farmers to test his research in real-world conditions.



Prodjinoto uses CRISPR-Cas9 gene editing technology to disable water channel proteins known as aquaporins. First, a helpful bacterium delivers a plasmid — a circular piece of DNA — into tomato plant cells. The plasmid carries a guide RNA, which directs Cas9 to the target spot in the genome, and the Cas9 protein, which cuts DNA. Inside the plant cell nucleus, guide RNA leads Cas9 to the *aquaporin* gene (3a), where Cas9 makes a precise cut in the DNA (3b). The plant tries to repair the break, but introduces small errors that disrupt the gene (3c), effectively switching it off. Researchers can now observe how the plant functions without that gene.

That's the basic overview. At a granular level, the research is painstaking and relies on a rapidly evolving, cutting-edge technique and complex data analysis.

CRISPR is a family of DNA sequences found in single-celled organisms such as bacteria. These sequences were first identified in 1987, though their role in adaptive immunity was understood only later. In 2012, biochemists Emmanuelle Charpentier and Jennifer Doudna demonstrated that by incorporating Cas9 — an enzyme that introduces double-stranded breaks in DNA — CRISPR could be reprogrammed to target and cut DNA sequences. The breakthrough earned them the 2020 Nobel Prize in Chemistry and was a key step in the development of the CRISPR-Cas9 editing tool.

Often referred to as "molecular scissors," CRISPR-Cas9 uses a guide RNA to direct Cas9 to a specific genetic location, where it induces cuts for deletion, editing or repair. Previously, geneticists relied on methods such as chemicals or radiation to induce mutations. CRISPR-Cas9 is quicker, cheaper and more accurate, with myriad research and medical applications.

To deactivate aquaporin genes in a tomato plant, Prodjinoto spent months analyzing genetic sequences and designing guide RNA molecules using specialized bioinformatics software. One of the biggest hurdles he faced was how to apply the two-step "Golden Gate" method, a technique that enables the simultaneous assembly of multiple DNA fragments into a single construct. Ido Nir, a CRISPR expert at Volcani and one of Prodjinoto's supervisors, along with HUJI molecular physiologist professor Menachem Moshelion, provided crucial assistance during this step.

Since starting his postdoc in March 2023, Prodjinoto has already pinpointed some aquaporin genes uniquely expressed in tomato roots or fruit, and others that are expressed in leaves and roots at the same time. "Further experiments will reveal the full extent of the effects of removing an aquaporin gene," says Prodjinoto. The second phase of his experiment involves phenotype and physiological analyses of transgenic plants under the stress of drought.

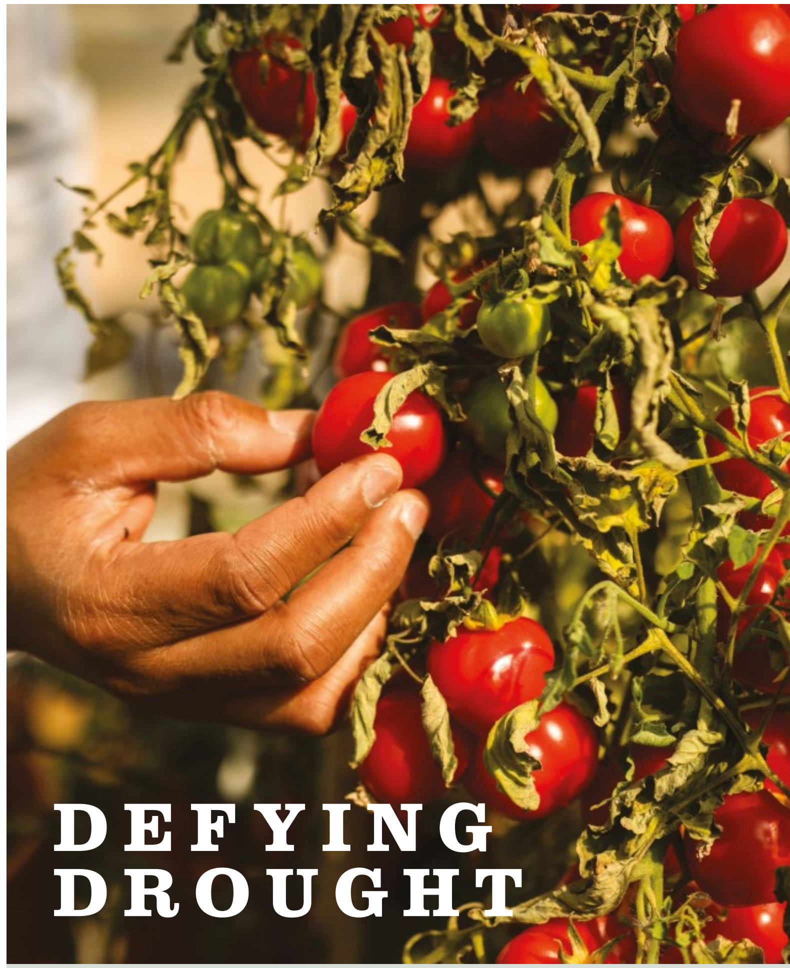
If everything goes according to plan, by the summer of 2025 Prodjinoto will have cultivated around 500 wild and transgenic tomato plants in a HUJI greenhouse and at the university's experimental farm on the edge of Rehovot. His monitoring equipment will collect data every few minutes, logging physiological traits such as biomass, growth rate, transpiration rate and stomatal conductance (a measure of how easily gases like water vapour pass through open stomata). The plants will be subjected to both control and drought conditions and observed throughout the vegetative, reproductive and fruiting stages of their growth.

Prodjinoto will analyze the extensive datasets using an advanced software platform to pinpoint the tomato plants that can best withstand drought conditions. This will involve measuring the relative expression levels of the 47 genes in various tissues and under the effects of different treatments using a technique known as quantitative real-time PCR; the technique amplifies a target DNA sequence while measuring the concentration of DNA in the tissue. Since all plants have aquaporins, the findings from this research could also be applied to other species.

"The integration of CRISPR-Cas9 and high-throughput phenotyping offers a powerful dual approach, like using a microscope and a telescope simultaneously," says Prodjinoto. "CRISPR enables precise editing of individual genes to study their functions, while phenotyping provides a comprehensive assessment of plant performance across diverse environmental conditions. This synergy could significantly accelerate the development of drought-resilient crops."

The dual "microscope-telescope" approach is perhaps the most innovative aspect of Prodjinoto's research. While it holds the potential to help feed people in our hotter, more crowded world, it also speaks to how we might adapt to and mitigate against the impacts of climate change more broadly — by looking at what's right in front of us and to the horizon at the same time. ▲●■

Since starting his research as an Azrieli International Postdoctoral Fellow in 2023, Prodjinoto has worked on developing transgenic plants he can monitor for fitness. Since all plants have aquaporins, his findings could be applied to other plant species.



DEFYING DROUGHT

Hermann Prodjinoto was seven years old when he moved with his mother to Godomey, a small community on the outskirts of Cotonou, Benin. That year brought little rain, and his farmer uncle lamented that the crops were struggling, with nothing to be done. Food prices soared, and life became challenging for Prodjinoto's family and countless others across Africa. Today, many regions on the continent are once again grappling with devastating droughts.

The memory of those struggles left a lasting impression on Prodjinoto. "When plants are thirsty," he says, "people are hungry." Drought not only reduces water availability but also increases soil salinity — a challenge he sought to address during his graduate school research. Now, as the first Azrieli International Postdoctoral Fellow from Africa, he is exploring innovative ways to help plants thrive in water-scarce environments. And he hopes to inspire a new generation of scientists to follow this path.

Whenever Prodjinoto returns to Benin, he speaks to students at local universities and collaborates with farmers to test his research in real-world conditions. Sharing knowledge and showing what is achievable are key parts of his mission. "When young people in Africa see someone who looks like them achieving these goals," he says, "it can ignite their belief that they, too, can succeed."

Prodjinoto adds, "The progress made by earlier scientists has paved the way for my work, and I'm determined to contribute research that not only helps people today but also serves as a foundation for future discoveries." ▲●■