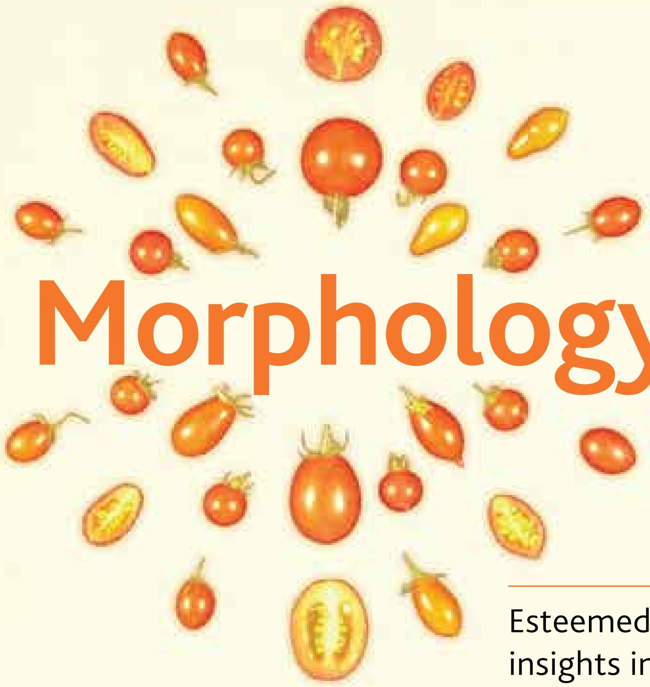

Morphology matters

Esteemed plant biologist **Dr Esther van der Knaap** shares insights into her career to date and highlights her current research projects into tomato fruit morphology

How did you develop an interest in this area of research?

I was born and raised at a greenhouse nursery in the Netherlands. My father ran a cut flower business of roses and orchids. Growing up, nearly everyone in my extended family worked in the cut flower production industry; as far as I can remember, I have always been around plants and greenhouses. In high school, I learned about DNA, RNA and proteins and was fascinated by the molecular aspects of biology – I wanted to know how plants grow and defend themselves from stress.

My undergraduate major was in plant pathology, and I completed a PhD in the area of plant molecular biology and physiology. When I started my postdoctoral studies, I was keen to combine genetics with molecular biology to understand plant growth and development from a quantitative perspective. This is where I started the research that now defines my career: the functional analysis of fruit shape and weight genes on tomato plant growth and development. Recently, I developed an interest in the evolutionary history of the tomato, from when it still had wild characteristics through selections of alleles and emergence of mutations resulting from domestication, that gave rise to the morphologically diverse germplasm. Although our main focus is on the tomato, we also work with other domesticated fruit and vegetable crops, including chilli pepper, aubergine and sweet/tart cherry.

What are you hoping to achieve as a result of your research into the discovery of genes and networks regulating tomato fruit morphology?

We are examining each gene in the tomato genome and assessing whether it impacts shape and size of the fruit. Now that we have identified several genes, we are keen to understand how they regulate shape and size at the molecular level. We are identifying co-regulated genes that show differences in gene expression resulting from fruit shape and size changes. In addition, we have modelled tomato fruit into eight different shape categories using various clustering methods and statistical analysis. By bringing these different approaches together, we hope to identify the gene regulatory networks that control fruit shape and size. In some cases, gene expression differences are very small, but, collectively, they show an impact of the entire genome on changes in organ shapes.

Why is the tomato a good model for the investigation of fruit development, organ patterning and the fundamentals of the plant growth process?

The tomato is a good model because of the genomic and genetic resources that are available: a relatively short generation time, a sequenced genome, mutant stocks, and a long history of research in fruit ripening and quality. Fundamental aspects of plant growth are studied in other plants as well, but the tomato is an excellent model due to its fleshy fruit and huge variation in fruit morphological characters.

How did you develop an interest in the plant hormone auxin? Has collaboration advanced your work in this area?

We think that fruit shape may be controlled in part by auxin, which is why we are putting an emphasis on this hormone. In this line of enquiry, we want to know in what way fruit shape genes impact auxin accumulation or signalling.

The projects related to fruit development and auxin are carried out alongside different collaborators based at other institutions who have excellent skills in this area. Cooperation is very important – we like to involve a broad base of research expertise in our studies, including undergraduate researchers from different disciplines such as computer science.

To what extent is a multidisciplinary approach important to the success of your research?

I collaborate with many other scientists, predominantly at research institutions. Collectively, by combining skillsets, we can really move biology forward. I also work closely with colleagues at the College of Wooster, a liberal arts college close to my campus of Ohio State University. I team up with biologists, but more importantly with computer science faculty and students, whose expertise is crucial for programming and data analysis projects. A large part of the Tomato Analyzer program was developed in this way, and we are currently collaborating on projects involving modelling of shape and analysis of promoter elements in co-regulated genes.

Unpicking the tomato

A series of research projects being carried out at **Ohio State University's Agricultural Research and Development Center** aim to provide valuable insights into the process of tomato morphology

THE TOMATO IS a hugely significant plant. In the US, it follows the potato as the second most important fruit or vegetable in economic terms, with the production of fresh and processed tomatoes bringing in over US $2 billion annually. Yet, despite the reliance upon the tomato industry by the US and other major producers – which include China, India, Turkey and Egypt – relatively little is known about its morphology and domestication.

Many currently believe that domestication of the tomato took place during two phases: the first in Ecuador and Northern Peru, and the second in Central America and Mexico. Wild tomatoes, unlike their cultivated counterparts consumed around the world, tend to be round and small, without much variety. Over the course of these two waves, it is assumed that the size and shape of the fruit changed from the small, resulting in the diverse range of tomatoes seen today. However, as knowledge in this area remains patchy, this has not been confirmed.

GETTING TO THE BOTTOM OF TOMATO MORPHOLOGY

Based at Ohio State University's Agricultural Research and Development Center, plant biologist Dr Esther van der Knaap leads a multidisciplinary team in a series of efforts to uncover more about the morphology driving the shape and size of a range of domesticated fruit and vegetable crops, with a particular focus on the tomato.

For logistical reasons, the morphology of fruit and vegetables is vital to the produce and fresh market industry. Fruit must be of an appropriate size and shape to avoid rolling off conveyer belts or becoming stuck in automated machines during harvesting and processing. Given the great variety in species being produced, morphology is particularly important in the case of the tomato. If growing tomatoes for canning, ellipsoid or rectangular-shaped fruit is preferable to flat or round types; if choosing a tomato to be used on a hamburger, a large, flat beefsteak is more appropriate than a cherry tomato.

It is this diversity – plus the abundance of genetic and genomic information available – that makes the tomato fruit an ideal model for van der Knaap and her fellow researchers to study. Furthermore, a greater understanding of the genetic factors determining the shape and size of tomatoes could have positive implications for growers, processors and consumers alike.

IT'S ALL IN THE GENES

A key component of van der Knaap's work is pinpointing the genes that regulate tomato fruit morphology. While advances in plant biology have brought about a better understanding of the genes that lay the basic groundwork of lateral organs such as leaves and fruit, further probing into the effect that such genes have on growth is required, as van der Knaap explains: "Our research into the shape and size of tomato fruit aims to shed light on this situation, and we hope our findings will go some way in explaining how plant organs form into different shapes and sizes".

The process by which the team is searching for these genes follows several stages. First, the researchers cross two different tomatoes with one another, before identifying the region of the genome containing the relevant gene using molecular markers. Once this has been picked out, van der Knaap and her colleagues home in on the area, until they are left with a small number of candidate genes. Finally, plant transformation and other types of genetics are employed in order to ascertain whether the genes in question actually affect shape and/or size of the fruit.

NETWORKING

The project has progressed to the next phase, where the researchers are looking to identify the molecular networks that may play a key role in the fruit's morphology. To achieve this, the scientists take four replicates and multiple fruit tissues from plants with different fruit shape and/or size genes, before sequencing their entire transcriptome. Modelling and statistics are then used in order to pinpoint the genes that are consistently differentially expressed between fruits of varying shape or size. When this stage

Tomato varieties carry mutations in SUN (S), FASCIATED (F), OVATE (O) and/or LOCULE NUMBER (LC) which is indicated by the letter in the fruit.

PLANTING TOMATOES

is completed, the team plans to look into the common transcription factors at the root of this phenotypic change.

THE IMPORTANCE OF SHARING DATA AND SOFTWARE

As an advocate of open data and exchanging of knowledge, van der Knaap has released various datasets generated from her studies into tomato morphology, and will continue to do so. The motive behind this is clear, as she states: "When we publish the data, we release all of the information so that others can use it in order to move their research forward".

Another tool that van der Knaap and her colleagues have shared is the Tomato Analyzer, a software package that facilitates the analysis of fruit and leaf morphology and colour developed by a group of applied mathematicians at the College of Wooster. The program, which was first built in 2003, is currently in its third incarnation and is available to download free of charge via van der Knaap's website (see Intelligence).

WIDENING PARTICIPATION THROUGH RESEARCH OPPORTUNITIES

Inspiring as many people as possible through her research is particularly important to van der Knaap, and she and her colleagues conduct a suite of public engagement activities in order for this to be achieved. Annual workshops for high school students are held at a community college with a view to spark an interest in plant genetics, food, agriculture and plant diversity not only among the students themselves, but also in their parents.

In addition, the group strives to offer a range of opportunities to students and early career researchers from a variety of disciplinary backgrounds and institutions. For example, the expertise of undergraduate computer science students from the College of Wooster is called upon for several modelling-related projects. Other valuable additions to van der Knaap's team include the graduate students and postdoctoral researchers based at the agricultural experiment station, who conduct genetics research and evaluations of plant growth and development. "Everyone has a speciality and together we hope to discover something unique about fruit diversity, while also exciting the broader public," van der Knaap enthuses.

> A greater understanding of the genetic factors determining the shape and size of tomatoes could have positive implications for growers, processors and consumers alike

A HIVE OF ACTIVITY

While tomato morphology is currently a strong theme for the lab, van der Knaap also leads and is involved in a plethora of other research endeavours extending beyond this particular fruit. She is the Principal Investigator on the new multidisciplinary Center for Applied Plant Sciences' pepper project, which aims to shed light on how domestication of chilli pepper led to adaptation and speciation of plant-associated organisms such as herbivores, parasitoids and microbes. Another area of interest for van der Knaap is the development of genetic tools such as markers for a range of fruits, and she has worked on projects that aim to build such resources for tomatoes and sweet cherries.

NEXT STEPS

Alongside her other commitments, van der Knaap will continue with her tomato morphology project, within which there is much more to discover. Once the team's research into molecular networks is complete, she plans for a detailed analysis of the expression of the fruit shape/size genes, the proteins they interact with and their location to follow.

The van der Knaap research team

INTELLIGENCE

DISCOVERY OF GENES AND NETWORKS REGULATING TOMATO FRUIT MORPHOLOGY

OBJECTIVES

- To identify and characterise genes controlling tomato fruit shape and size
- To discover the underlying molecular networks that control fruit morphogenesis

KEY COLLABORATORS

Co-Principal Investigators:

Brian McSpadden Gardener, The Ohio State University/Ohio Agriculture R&D Center)

Carmen Catalá, Boyce Thompson Institute, New York, USA

Sofia Visa, The College of Wooster

Collaborators:

Carri Gerber, The Ohio State University/ Agriculture Technical Insitute

Yuji Kamiya, RIKEN, Yokohama, Japan

Kazuki Saito, RIKEN, Chiba University, Chiba, Japan

Simon Gray; John Ramsay, The College of Wooster, Ohio, USA

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ESTHER VAN DER KNAAP is an Associate Professor in Horticulture and Crop Science at The Ohio State University and affiliated scholar at the College of Wooster. Van der Knaap received her BSc and MSc in Plant Pathology from Wageningen University in the Netherlands in 1990, PhD in Genetics at Michigan State University in 1998, and postdoctoral training in Plant Breeding at Cornell University in 2001. Her research interests focus on the functional analysis of genes that control plant organ shape and weight using tomato fruit as the system.