**////Title:** **Investigating Fructans to Understand How Plants Can Survive Harsh Environments**

**////Standfirst:**

The molecules within plant tissues can tell us about how they can withstand harsh environmental conditions. The *Agave* [ah-gah-vay] *tequilana* [tuh-keel-AH-nah] plant, native to Mexico, has a high concentration of fructan molecules throughout its tissues. Alongside his colleagues, Dr José Ordaz-Ortiz [hoe-ZAY or-dass or-teece] at the Center for Research and Advanced Studies of the National Polytechnic Institute in Mexico, combines several powerful analytical techniques to better understand the role that these fructans play in plant biology.

**////Main text:**

Metabolomics is the study of the chemical processes that occur within a biological system, such as the cell of a plant. Each chemical process within a cell takes in molecules, such as water and nutrients, and then metabolises them to produce a range of small molecules known as metabolites.

In plants, photosynthesis is the simplest of these processes – using energy from sunlight to convert carbon dioxide and water into oxygen and glucose. Whilst some of this glucose is used directly as an energy source for the plant, some is also converted into starch, which acts as an energy store for when there is little sunlight. However, some species of plants, such as *Agave tequilana*, also convert this glucose into fructans. In fact, *Agave tequilana* contains some of the highest levels of fructans of any plant species. These fructans are the raw material for that much-loved Mexican spirit, Tequila.

However, fructans aren’t only useful in the production of tequila. Because of their high water-solubility, fructans are believed to play a role in the ability of the plant to regulate its water usage. This helps the plant to withstand the hot temperatures and dry conditions of the Mexican desert. In most plants, fructans are primarily present in the roots and bulbs, but they are present throughout the leaves and stems of *Agave tequilana*.

In a unique study, Dr José Ordaz-Ortiz and his colleagues used a technique called mass-spectrometry imaging to create a concentration and structure profile of the various fructans within *Agave* *tequilana*. Where previous methods have involved destroying plant matter to extract fructans, therefore limiting their traceability, this new technique allowed the team to pinpoint specific fructan molecules and determine their distribution throughout the plant.

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A simple technique for investigating the starch content of a plant is to peel a thin layer from its surface and stain it with a chemical that highlights the presence of starch. Because many starches can be broken down into fructans, staining was the starting point for Dr Ordaz-Ortiz and his colleagues.

To compare the distribution of fructans within the plant, the team examined two main areas. Firstly, they investigated the stem, which they sliced into six sections – both lengthways and horizontally – to produce a 3D profile of the fructans. Secondly, they took samples from the rhizomes – the part between the stem and the roots where new plants branch off. They studied this area to develop an understanding of how the mother plant provides nutrients for the growing offshoots.

The results of the team’s experiments showed that starch is only present in a thin layer on the periphery of the stem where it provides strength, but fructans were present across the whole of the stem and within the rhizomes. This implies that the mother plant can transfer fructans into the rhizomes, promoting the growth of new plant tissue.

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In order to identify the fructans present in the plant, the team used mass-spectrometry. Traditional mass-spectrometry is a technique that is typically carried out on small molecules, which can be easily ionised. However, most biological molecules, including fructans, do not typically have this property. Therefore, the team also used matrix-assisted laser desorption ionisation, referred to as MALDI. This technique enables the chemical analysis of a substance that cannot be readily ionised, by mixing it with a reactive species known as a matrix, which helps to ionise the substance.

The team prepared a sample for MALDI by pressing the tissue segment onto a nylon membrane, leaving behind a thin layer of cells. The MALDI analysis then produced a computerised image which showed the masses of different molecules present in the sample. Through this, the researchers revealed that the rhizomes are dominated by smaller fructans while the core of the stem features larger fructans.

The team also used another mass spectrometry method to estimate the shape and size of the fructan molecules. They then carried out computer modelling combined with Ion Mobility Spectrometry to determine the exact structure of a large range of different fructans. Finally, the researchers explored the plant’s genetics, and found that the enzymes responsible for breaking down fructans are present in high concentrations in the plant rhizomes, while those responsible for forming larger fructans are more likely to be found in the stem.

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Overall, this study has shown that it is possible to build an accurate profile of fructans within a plant, through a clever combination of analytical techniques. The team’s approach to combining spectrometry, modelling and genetic studies to determine detailed information about biological molecules within an organism could also be readily translated to other metabolomic studies.

Because fructans regulate water transfer and usage within plants, the knowledge obtained in this study could aid the development of drought tolerance in food crops through genetic engineering. In addition, the cultivation of hardy Agave species could give arid regions an economic boost. Such regions are becoming dryer and are growing in size as climate change accelerates.

As well as in the production of Tequila and Mescal, there are many potential uses of these fructan-rich plants. Because small fructan molecules have a sweet taste but cannot be properly digested by enzymes in the human body, they hold potential as a low-calorie sweetener. There is also evidence that some fructans can stimulate the immune system, while others are known to have prebiotic qualities, promoting the growth of healthy gut bacteria. Finally, Agave plants are also recognised for their use as a potential biofuel source, helping to reduce humanity’s reliance on fossil fuels.

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This SciPod is a summary of the paper ‘Localization and composition of Fructans in Stem and Rhizome of *Agave Tequilana* Weber var. azul’, in *Frontiers in Plant Science*. [doi.org/10.3389/fpls.2020.608850](https://doi.org/10.3389/fmicb.2020.01520)

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