Mystery behind striped barley solved

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Picture of an albostrians barley. Credit: Josef Bergstein / IPK Gatersleben

Plants with green leaves and stems are a common sight and are one of the most natural things on earth. But when considering that this colouring is achieved by small chlorophyll-filled organelles called chloroplasts, distributed within plant cells, where they utilise their green pigmentation to convert solar energy into chemical energy, this green colouration no longer seems to be such a trivial thing. Because of their fundamental role in plant biology, chloroplasts and their ability to colour plants have long been the focus of intense research. Specifically, genetically impaired chloroplasts which no longer or only partially express pigments, are used to identify genes and understand the molecular mechanisms within plant cells.

One such plant which displays the effect of mutated chloroplasts is called Albostrians barley. Instead of growing green leaves, this grass plant is patterned with green-white stripes, an effect called variegation. Even though scientists have been using albostrians mutants in order to investigate plant cellular mechanisms, the underlying gene behind this variegation effect was unknown until recently. A group of scientists from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben together with researchers from the Humboldt University Berlin and KWS LOCHOW GmbH have now identified the underlying gene, HvAST, which is causing this albostrians phenotype, spurring novel insights into chloroplast development.

Chloroplasts are green, chlorophyll-filled plastids found in plant cells. These plant organelles play a pivotal role for life on earth, as they perform photosynthesis, thus enabling plants to develop and grow. Chloroplast biogenesis is the process in which chloroplasts mature in plant cells from so-called pro-plastids. This process can be affected by external factors, such as temperature, and relies heavily on the synergic expression of proteins encoded within the nuclear and the plastid genome of the plant. As the plastid genome encodes only a fraction of proteins required for chloroplast biogenesis, the nuclear genome delivers the vast majority. Consequently, mutations within the nuclear genes can easily result in defective chloroplasts.

Whilst functioning chloroplasts are of highest importance in nature, impaired chloroplasts are of great value within research. Mutants which lead to aberrations in the colouration of plants can be used as genetic tools to identify genes involved in chloroplast biogenesis and to understand related molecular processes within plants. Especially mutations which lead to variegation, the appearance of differently coloured (white, yellow, green) areas on plants, are of great interest for plant researchers. This phenotype is caused by the presence of both normal and defective chloroplasts in different sectors of the same plant tissue, and scientists are able to utilise this during research, for example when investigating communication between cell organelles or when examining the molecular mechanisms behind variegation itself.

Albostrians barley, with its green-white striping, is a well-known example of a plant mutant displaying variegation. As a model plant, it has helped broaden the field of chloroplast biology during previous research work. However, the clarification of the mechanism leading to the albostrians-specific phenotype of variegation had previously been hindered by the fact that the causal underlying gene was unknown. Scientists from various research groups of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben) in cooperation with researchers from the Humboldt University Berlin and KWS LOCHOW GmbH have now identified the responsible gene.

Utilising positional cloning, the ALBOSTRIANS gene was identified as the CCT-domain gene HvAST. The scientists validated the functionality of the gene twice, first by inducing a knock-out by chemical mutation and detecting the responsible mutated gene through TILLING and then by inducing mutations through RNA-guided Cas9 endonuclease mediated precise gene editing. Further, HvAST was found to be a homolog of the CCT Motif transcription factor gene AtCIA2 of the plant Arabidopsis thaliana. However, while AtCIA2 is reported to be involved in the expression of nuclear genes and thus plays a key role in chloroplast biogenesis, the researchers surprisingly found that CCT-domain containing protein HvAST was localised to plastids in barley and found no clear evidence of nuclear localisation. Nevertheless, HvAST presumably has an important function for plastid ribosome formation during the early embryo development and consequently for chloroplast development.

"Since the early 1950s, scientists have studied the variations of pigmentation, as this phenomenon allows insights into important gene functions and regulations and therefore into the basics of the genetics of living organisms. With the present work, we have succeeded in identifying one of the key genes involved in this process", says Prof. Dr. Nils Stein from the IPK and corresponding author of the team. Dr. Viktor Korzun leading researcher at KWS SAAT SE, also involved in the study continues: "The new insight into the role of this CCT-domain-containing protein, and more importantly, the identification of the gene underlying the "albostrians" phenotype can now be followed up by new in-depth investigation of the mechanisms of the barley mutant, and is likely to foster new research in the area of leaf variegation and chloroplast development."

More information: Mingjiu Li et al, Leaf Variegation and Impaired Chloroplast Development Caused by a Truncated CCT Domain Gene in albostrians Barley, *The Plant Cell* (2019). DOI: 10.1105/tpc.19.00132

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