

A guide through the genome of crops





BZR1 regulatory network in maize and *Arabidopsis*. **a** Distribution of ZmBZR1 binding around transcribed genes. Frequency of ZmBZR1 binding peaks up to 10 kb up- or downstream of TSS or TTS and intra-genic, respectively. **b** ChIP-seq identified ZmBZR1 binding in proximity of putative targets repressed (*BR6ox2/BRD1*), induced (*IAA19*) or not controlled by BR (*CNX5*). Black line shows normalized BZR1 ChIP reads (reads per genome coverage, RPGC) and red line depicts the negative control (non-transgenic siblings). Genes are depicted in blue; black arrows indicate direction of transcription. **c**, **d** Significantly overrepresented ZmBZR1 binding motifs, BRRE (CGTG[C/T]G) and G-box (CACG[A/T]G) (**c**) as well as BRRE significantly co-localizing secondary motifs



for TCP TF class I (GG[A/C]CCA) and class II (GTGGGC) (**d**) determined by GEM. **e** Direct and indirect targets of ZmBZR1. Shown is the overlap of BZR1 ChIP-seq and RNA-seq of the BR-deficient *brd1* mutant +/– BR. **f** Conservation of the BZR1 targets between *Arabidopsis* and maize (*Arabidopsis* orthologs). **g** Conservation of *Arabidopsis* and maize (*Arabidopsis* orthologs) BR up- and downregulated genes. **h** Overlap of orthologous BR-responsive (B) and non-responsive (N) BZR1 target genes (T) and non-target genes (N) in *Arabidopsis* (At) and Maize (Zm). Color-coding indicates fold enrichment compared to random expectation (blue, low enrichment to red, high enrichment). e^{Numbers} indicate *p*-values of significance of this enrichment assuming a hypergeometric distribution. **i** Heatmap of direct orthologues target genes of BZR1 in *Arabidopsis* and maize induced (green) or repressed (red) by BR. Overall, 65% of 1:1 orthologs and 72% of 1:2 copy orthologs of direct BZR1 targets between *Arabidopsis* and Maize, respectively, showed the same direction of BR regulation. Credit: *Genome Biology* (2023). DOI: 10.1186/s13059-023-02909-w

Plants show enormous variety in traits relevant to breeding, such as plant height, yield and resistance to pests. One of the greatest challenges in modern plant research is to identify the differences in genetic information that are responsible for this variation.

A research team led by the "Crop Yield" working group at the Institute for Molecular Physiology at Heinrich Heine University Düsseldorf (HHU) and the Carnegie Institution of Science at Stanford has now developed a method to identify precisely these special differences in genetic information.

Using the example of maize, they demonstrate the great potential of their method in the journal *Genome Biology* and present regions in the maize genome that may help to increase yields and resistance to pests during breeding.



The blueprint of all organisms is encoded in their DNA. This includes the genes that encode the proteins and determine an organism's inherent characteristics. In addition, there are other important sections of the DNA, in particular the regions that control the regulation of genes, i.e. when, under which conditions and to what extent the genes are activated.

Compared to the genes, however, these <u>regulatory regions</u>—also known as "cis elements"—are difficult to find. Changes in precisely these DNA elements are largely responsible for the differences between organisms—and thus also between different plant varieties.

In the past few decades, researchers have discovered that the regulatory regions are the binding sites of specific proteins. Known as <u>transcription</u> <u>factors</u>, they determine when and for how long genes are activated.

Co-corresponding author Dr. Thomas Hartwig, who heads the Crop Yield research group at HHU's Institute for Molecular Physiology and the Max Planck Institute for Plant Breeding Research (MPIPZ) in Cologne explains, "Finding the few variations that are key to changing traits such as pest resistance among the millions and millions of noncausative genome differences is the ultimate search for a needle in a haystack."

"Unlike protein-coding genes, regulatory sites usually cannot be identified based on the sequence alone. This makes them very difficult to pinpoint. Our method uses <u>hybrid plants</u> to measure the direct effects of variation in DNA sequence on transcription factor binding," says lead author Professor Dr. Zhi-Yong Wang from the Carnegie Institution for Science.

The study was developed in a cooperation with researchers from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben as well as from the University of Nebraska-Lincoln and



Iowa State University, U.S..

Using hybrids, i.e., the first generation of plants created by crossbreeding two varieties, the research team can compare which regulatory regions differ across the entire genome. Co-author Dr. Julia Engelhorn says, "Our analytical method allows us to measure precisely whether transcription factors bind more to the maternal or paternal genome." This procedure has also enabled the team to identify thousands of differences associated with traits, such as yield and pest resistance in maize.

The technology was demonstrated for a transcription factor in the brassinosteroid pathway, a hormone related to growth and disease. Institute director Professor Dr. Wolf B. Frommer says, "The team has identified thousands of genomic variations that can explain why one variety of maize behaves differently in terms of its yield or resistance to disease. Moreover, the team was able to show that these differences are almost equally genetic and epigenetic." The latter describes processes that influence gene activity without being encoded in the DNA sequence itself.

One central result of the study is a list of more than 6,000 genome regions that can be targeted for plant breeding. These may include, regions through which positive traits are expressed in certain maize varieties that others plants lack.

Hartwig says, "Knowing where in the genome modern breeding methods can be applied to transfer characteristics from certain varieties to others is of great importance to biotechnology. Our study may serve as a guide on how to find these interesting <u>genome</u> regions."

Professor Frommer adds, "The study findings lay the foundation for using modern techniques to cultivate new varieties of maize by skillfully



combining the optimal variants."

More information: Thomas Hartwig et al, Hybrid allele-specific ChIPseq analysis identifies variation in brassinosteroid-responsive transcription factor binding linked to traits in maize, *Genome Biology* (2023). DOI: 10.1186/s13059-023-02909-w

Provided by Heinrich-Heine-Universität Düsseldorf

Citation: A guide through the genome of crops (2023, May 19) retrieved 27 April 2024 from <u>https://phys.org/news/2023-05-genome-crops.html</u>

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