Institute of Crop Science
Chinese Academy of Agricultural Sciences

MiR528/Laccase

Maize lodging resistance

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

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Patent Literature

Publications: WO/2019/080727, EP 3701033, US 2020/0283786, IN 202017021724A, CN 111630171A, BR112020008016-0, CL 1074-2020

Lodging has long been an intractable problem for corn breeders and a cause of sometimes catastrophic yield loss for growers. Because lodging happens in a chaotic, uneven manner under real field conditions it is difficult to measure accurately and reliably, particularly in early generations of selection. Consequently, selection for resistance to both stalk and root lodging is inefficient, often relying on proxy and primitive methods to make some approximation where nothing else is possible. As a result elite new material is often found wanting when the environmental conditions prevail that precipitate lodging on a dramatic scale. This preponderance for lodging is exacerbated when crops are grown under intensive fertilization conditions, as is common in modern practice. However, molecular mechanisms affecting lodging in maize are poorly understood and the trait has remained intractable to direct genetic strategies for enhancement.

The group of Wen-Xue Li at the Institute of Crop Science, Chinese Academy of Agricultural Sciences in Beijing, have found that that the maize microRNA ZmmiR528 is mainly expressed in vascular tissue, in particular in the phloem, and interestingly its expression is increased with increasing nitrogen supply. They therefore decided to analyse its possible role on modulating lignin biosynthesis. They found that ZmmiR528 specifically targets two maize laccases, ZmLAC3 and ZmLAC5, reducing their abundance. The two laccases are involved in affecting lignin levels in vascular tissue. Laccases are copper-containing glycoproteins and provide an essential function, with peroxidase, in lignin polymerization. Lignin, the second most abundant biological polymer after cellulose, is centrally important for stem stiffness and strength, not to mention resistance against pests and pathogens. The ICS group found that knockdown of ZmmiR528 or overexpression of *ZmLAC3* or *ZmLAC5* significantly increased lignin content and rind-penetrometer resistance of maize stems. Conversely, the overexpression of miR528 in transgenic plants (“OE lines”) caused reduced lignin content and plants that were more prone to lodging, particularly under conditions of plentiful N supply.

There are two miR528 copies in maize and they are relatively highly expressed. Therefore, in order to study the loss-of-function “knock-down” phenotype, rather than generating genome edited disruption mutants, the inventors used a target mimicry (“TM”) approach expressing short tandem mimics of ZmmiR528a and ZmmiR528b in transgenic lines. This also has the advantage of providing a dominant phenotype. Lignin content in the TM lines was 1.5 times greater than in the wild type control lines. The TM lines also had significantly higher rind penetrometer resistance than WT, while OE lines were significantly lower. Moreover, cell number was lower and cell diameter larger in the phloem of OE lines over-expressing ZmmiR528 compared to both WT and TM lines, suggesting that phloem cells are arranged more loosely in OE lines. In a simulated lodging experiment, plants were grown in 10kg soil in pots for 70 days under high fertilization conditions. OE transgenic lines were more prone to lodging than WT or TM plants (Fig 1). The ICS group made hybrids with either the WT lines (CZZCO1) as one parent (L) or a miR528 TM knockdown line as one parent (R) and tested these in field trials (Fig 2), which show that miR528 knockdown improves lodging resistance under field conditions.

As well as modifying levels of ZmmiR528 to affect LAC abundance and consequently lodging resistance, the ICS researchers explored the alternative approach of directly overexpressing Laccase in transgenic plants. Overexpressing *ZmLAC3* (or *ZmLAC5*) caused increased lignin content of 11-20% in mature stems, compared to WT (Fig 3). Rind penetrometer resistance was also higher in OE *LAC* lines.

Further characterization of the molecular interactions revealed that the promoters of ZmmiR528a/b contain binding sites for the TGA1/4 and NAC4 transcription factors that are key regulators of nitrate response. Also, ZmmiR528a/b are shown to directly cleave ZmLAC3 and ZmLAC5.

References:

Sun Q, Liu X, Yang J, Liu W, Du Q, Wang H, Fu C and Li W-X (2018). MicroRNA528 Affects Lodging Resistance of Maize by Regulating Lignin Biosynthesis under Nitrogen-Luxury Conditions. *Mol. Plant*: 11; 806-814.

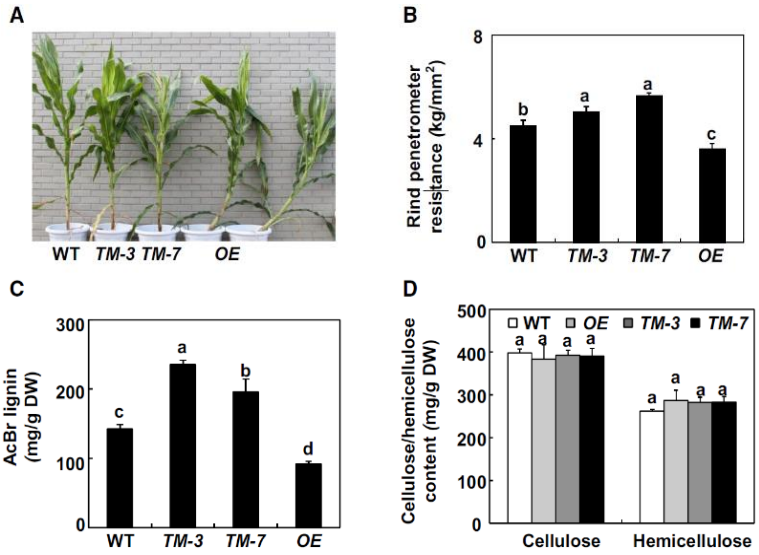

Fig 1 Overexpression of miRNA528 increases susceptibility to lodging in soil-grown maize (A). Conversely, knockdown of miR528 by target mimicry (TM) increases lignin content (C) and rind penetrometer resistance (B). Other structural components such as cellulose and hemicellulose are not affected (D). Measurements taken from 3rd internode at V9 growth stage.

Fig 2 WT Hybrid (Top) vs TM-7 Hybrid (Bottom). Hybrids made with either the WT lines (CZZCO1) as one parent (Top) or a miR528 TM knockdown line as one parent (Bottom), show that miR528 knockdown improves lodging resistance under field conditions

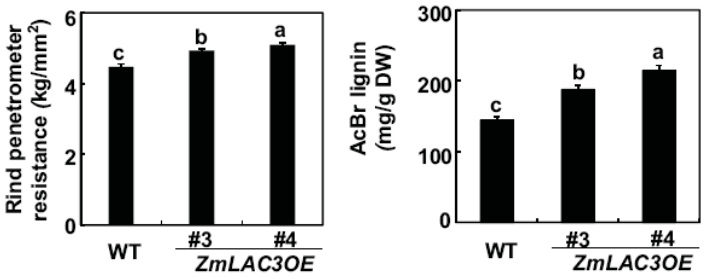

Fig 3 Overexpression of Laccase increases lignin content and increases rind penetrometer resistance on soil-grown maize

Knockdown of miRNA528 was achieved here by target mimicry. Mutation or genome editing could also be used although both miRNA528 copies would need to be targeted. A further strategy would be to modify the miRNA target sites in *ZmLAC3* and *ZmLAC5* to make them resistant to miRNA degradation. Screening for natural variation in levels of miRNA528 and/or *LACs* could also be employed to address this trait enhancement opportunity, either directly or by molecular marker approaches.

The ICS team also found that several ZmPALs (PAL-Phenylalanine Ammonia Lyases) are much more highly expressed in ZmmiR528 TM knockdown lines than in WT or the OE lines. PALs not only catalyse the first step in monolignol synthesis from phenylalanine but also are a key link in the phenylpropanoid-nitrogen cycle.

MiRNA528 is a monocot-specific miRNA. Although the mature sequence of miRNA528 is the same in rice and maize, the function differs in these two species. In rice miR528 negatively regulates virus resistance by cleaving ascorbate oxidase mRNA, thereby reducing AO-mediated accumulation of ROS (Wu et al 2017, Nat Plants 3:16203).

While the most important application of these findings is to provide methods for improving lodging resistance of maize, the overexpression of ZmmiRNA528, reducing lignin deposition, could have applications in forage or biofuel.

In summary:

- **A biotechnological approach to enhancing root lodging is provided**
- **A range of different strategies possible involving either miRNA528 or LACs**
- **Possible forage and biofuel uses**

The miR528/Laccase technology is patented by PBL on behalf of ICS CAAS. **For more information or licensing interest, please contact PBL.**