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Autoimmune mutants reveal new immunity pathways in maize

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The plant immune system includes constitutive and induced responses, when the strict fine-tuning regulation is disrupted autoimmunity may occur, i.e. spontaneous initiation of plant defense response affecting plant performance. In a screening to look for developmental affected maize mutants we identified *narrow odd dwarf* (*nod*) and *Liguleless narrow* (*Lgn*); surprisingly, transcriptome analysis showed overrepresentation of defense related gene categories, suggesting autoimmunity in these mutants. Proteomic analysis of NOD and LGN immunoprecipitated complexes showed interaction with exocyst components and MAPKs, and NOD with LGN interaction, BiFC experiments showed plasma membrane localization of NOD, LGN and some interactors relating these proteins with MAPK signaling pathways. NOD is the maize ortholog of the Arabidopsis MCAs (*Mid-Complementing Activity 1* and *2*), which have been reported forming a calcium import channel, based on the 3D structure and the MLKL domain, recently MCA2 was proposed to be a hNLR protein. In addition, NOD was identified interacting with *P. maydis* effectors in a Y2H screen, suggesting that NOD may be an atypical NLR. To test whether NOD can produce cell death, we did transient expression in *N. benthamiana*, showing that NOD N-terminus domain is enough to produce cell death. To explore the *nod* and *Lgn* involvement in pathogen response we are using *Setosphaeria turcica* to infect mutants in different genetic backgrounds. We found differential expression patterns of pathogenesis-related genes in B73 and Mo17, suggesting that those proteins are preventing PR genes induction depending on genetic background. Next experiments with fungal effectors will give information about NOD and LGN function at molecular level during pathogen infection in maize.

Gene / Gene Models described: *nod, Lgn1*; Zm00001eb004320, Zm00001eb382080
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Challenges and insights from establishing a maize transformation laboratory

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Maize genetic transformation, especially that mediated by Agrobacterium tumefaciens, is a crucial method for creating genetically modified varieties. Besides its extensive utilization for generating transgenic events, it is currently the method of choice to deliver CRISPR/Cas9 machinery into maize cells, allowing the production of genome-edited genotypes. In recent years, significant advancements in maize transformation protocols have been made. These developments have gained attention from both academic and industry organizations, resulting in the emergence of several public and private maize transformation laboratories and facilities worldwide. In 2019, we established maize transformation capabilities at the Genomics for Climate Change Research Center (GCCRC) in Campinas, Brazil, as part of a plant biotechnology pipeline using maize immature zygotic embryos of the model temperate inbred line B104. We observed a significant increase in transformation efficiency after incorporating the utilization of ternary vectors and morphogenic regulator genes (MR). The “altruist” method increases transformation efficiency and the number of fertile plants by avoiding the adverse effects caused by defective MR excision when using a single vector for the target and MR genes. With these optimizations, we can now transform and edit tropical maize lines more efficiently than B104. Seasonal variation has a significant impact on embryo production, which is highly correlated with transformation efficiency. Currently, we are testing a leaf-based protocol, which has shown promising results in overcoming this issue.

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