

Mathematical modeling is an effective approach in predicting the temporary expression pattern of the nitrate transporter gene *NRT2.1*

Nitrogen (N), a constituent of many biomolecules such as nucleic acids and proteins, is an indispensable element for plants. Among the major forms of N, nitrate is prevalent under oxidative environments and its availability is closely related to plant growth. However, excessive nitrate uptake leads to increased energy use and reduced pathogen resistance. Thus, plants fine-tune nitrate uptake by regulating the expression of the gene encoding a major nitrate transporter, *NRT2*. Although it is important to manipulate the expression of *NRT2* to modify N use, intuitively understanding key components for such a modification is difficult, especially when the gene is under a complex regulation. For designing plants with optimized N use and increased resilience, it is important to quantitatively understand the changes in the response caused by changes in the regulatory pattern. This study aimed at elucidating important regulatory factors for *NRT2* by comprehensively analyzing its regulatory system via mathematical modeling.

Temporary changes in the expression of Arabidopsis *NRT2.1* (a member of the *NRT2* family) were fitted to an ordinary differential equation to determine coefficients, and a mathematical model describing the temporary expression pattern of *NRT2.1* and other related molecules was developed (Fig. 1). The model predicted that the absence of negative regulation of *NRT2.1* by NIGT1, a transcriptional repressor, decreases the stability of *NRT2.1* expression under a wide range of activity of NLP, which induces the expression of *NRT2.1* and *NIGT1* genes in the presence of nitrate (Fig. 2). This hypothesis was further validated experimentally using mutant plants lacking the regulatory pathway from NIGT1 to *NRT2.1*; the expression of *NRT2.1* was stable under a wide range of nitrate concentrations in the wild-type plants, whereas the expression of *NRT2.1* was greatly affected by nitrate concentrations in the mutant plants (Fig. 3).

The quantitative description of the temporary response pattern related to N use provides clues on which regulatory component should be altered for a certain desired response. Since a similar regulatory pattern of *NRT2* is conserved in other plant species including rice, this mathematical modeling is likely to be effective in other plant species. This approach is also applicable to other traits and other plant species, especially when the trait is under a complex regulation. This approach is useful for designing plants with favorable traits and accelerating smart breeding. Further understanding of molecular mechanisms and the expansion of more fundamental data will be helpful to accelerate the applicability of this approach.

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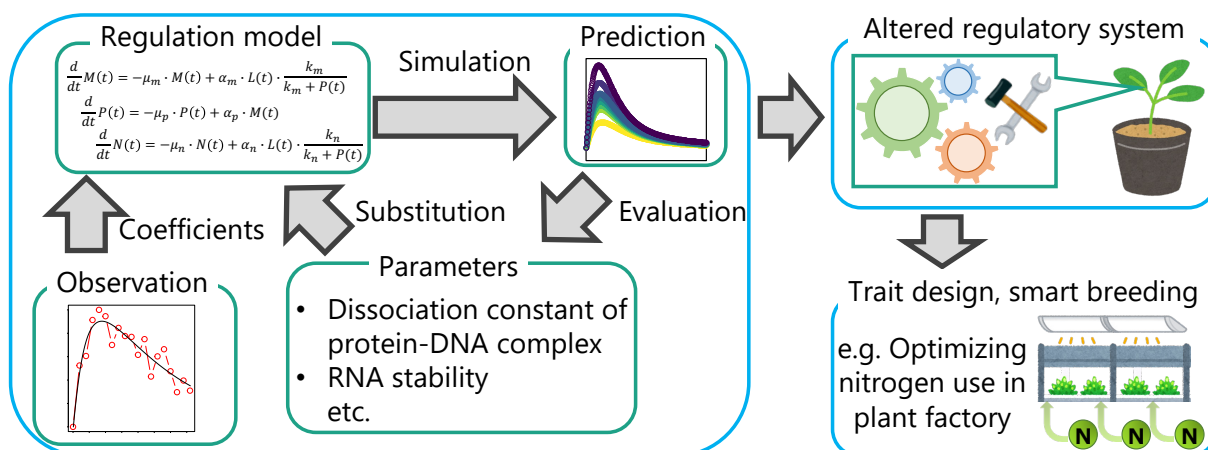


Fig. 1. Concept diagram of the development and use of a mathematical model

Coefficients obtained from experimental data are used to construct the regulation model. The effect of each parameter on the response pattern is evaluated by simulations. The results of simulation shall be used to alter regulatory systems and design crops conferred with a desirable trait.

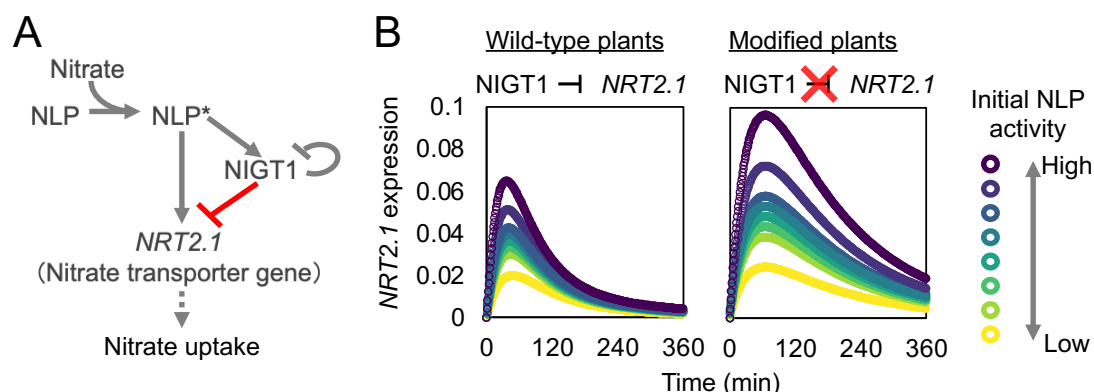


Fig. 2. Regulatory systems involved in nitrate use and prediction of their behavior

(A) Regulatory system involved in nitrate use. Arrow and T sign indicate promotive and suppressive effects, respectively. NLP* indicates activated NLP protein in the presence of nitrate. (B) Predicting the temporary expression pattern of *NRT2.1* in wild-type plants (left panel) and modified plants (right panel) lacking NIGT1 regulation (red sign in A).

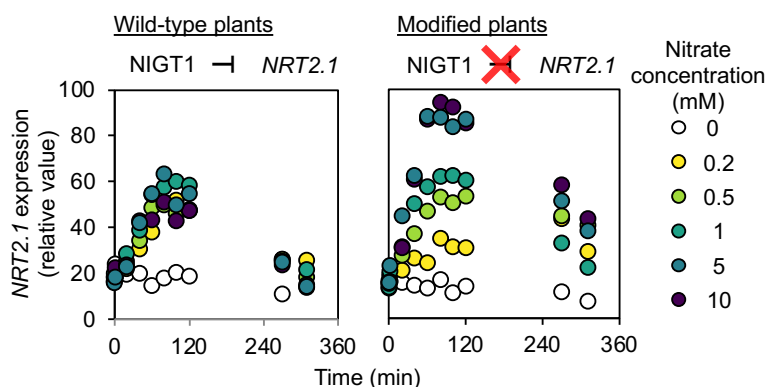


Fig. 3. Experimental validation of the simulation result

Experimentally determined expression levels of *NRT2.1* in the presence of different nitrate concentrations are shown for wild-type plants (left panel) and modified plants (right panel) lacking NIGT1 regulation.