BNI-enabled wheat is nitrogen-efficient and maintains productivity

The amount of nitrogen fertilizer used in modern agriculture is enormous, amounting to about 120 million tons worldwide. However, most of it (about 70%) is not absorbed by crops and is leached out from farmland, making farmland a source of pollution to the aquatic environment and emission of nitrous oxide (N_2O), a global warming gas with a greenhouse effect up to 298 times greater than that of carbon dioxide. The release of excess nitrogen into the environment is related to nitrification in the soil. Nitrification is a microbial oxidation reaction from ammonia-form nitrogen (NH_4^+-N) to nitrate-form nitrogen (NO_3^--N), which is an important pathway in the nitrogen cycle of the earth. $NH4^+$ is retained in the soil and does not migrate much, while NO_3^- is weakly bound to the soil and is highly mobile, so it easily leaches out of the farmland. Nitrification in agricultural soils is highly active due to a large amount of industrially fixed nitrogen fertilizer applied, so the conversion to NO_3^-, which cannot be retained by the soil, and its leaching into the hydrosphere proceed rapidly, and N_2O is released into the atmosphere during the process (Fig. 1 left).

Therefore, if the nitrification rate of agricultural soil can be suppressed, it could be an effective means of solving the problem. Biological Nitrification Inhibition (BNI) is the process by which crops themselves secrete substances that inhibit nitrification. BNI technology that utilizes BNI can both maintain and increase crop yields with less nitrogen fertilizer input and reduce environmental impact. By inhibiting nitrification, crops have more opportunities to absorb nitrogen, which allows reducing NO_3^- leaching and N_2O release. The introduction or enhancement of BNI capacity through breeding can be expected to both reduce agricultural greenhouse gas emissions and nitrogen fertilizer application.

We have been investigating the BNI potential of wheat, a major cereal covering the largest area among food crops, and developed BNI-enabled wheat by introducing superior BNI capacity from *Leymus racemosus* into high-yielding wheat varieties through chromosome engineering tools. Substitution of *Leymus racemosus* chromosome N short-arm with wheat chromosome 3B introduced BNI capacity, and the resulting line was further back-crossed with high-yielding varieties. (Fig. 2) The BNI-enabled variety, "BNI-Munal," showed around 2–5 times higher BNI capacity than the parental variety, "Munal." This high-yielding background "BNI-Munal" showed suppression of nitrifying microorganisms in rhizosphere soil, resulting in the lowering of soil nitrification rate and N_2O emission (Fig. 3); therefore, the environmental load by agriculture caused by nitrogen fertilizer can be reduced. "BNI-Munal" also showed efficient use of NH_4^+ in terms of nitrogen assimilation and soil organic nitrogen. "BNI-Munal" showed a significantly higher yield than Munal, and a 60% reduction in nitrogen application (from 250 to 100 kgN/ha) did not show a difference in yield between BNI-enabled and parental variety, hence the grain protein content (and bread-making quality) also did not change (Fig. 3). Further improvement in BNI capacity can be made by reducing the N chromosome short-arm and elucidating the mode of action, which is ongoing.

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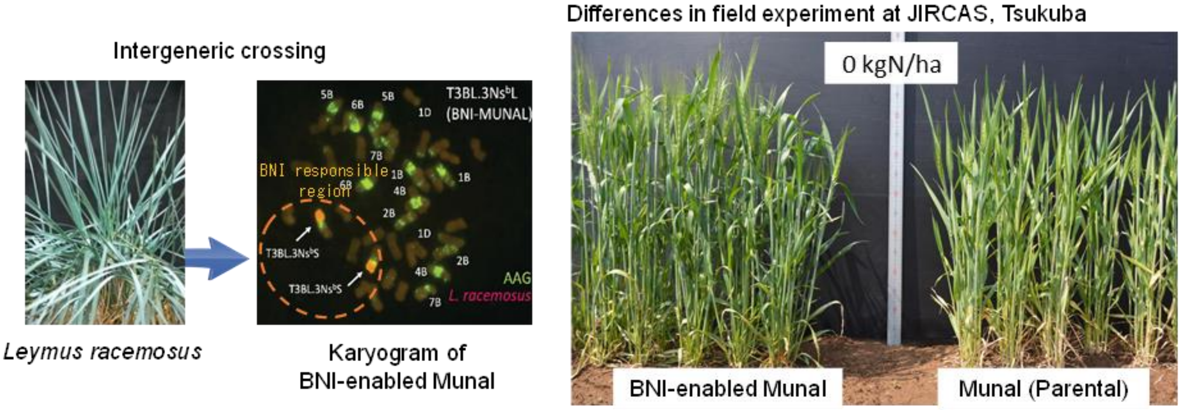
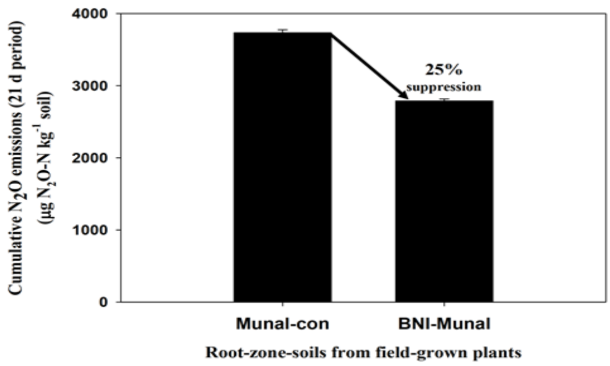


Fig. 1. BNI-enabled wheat with *Leymus racemosus* N chromosome (ex. BNI-Munal)



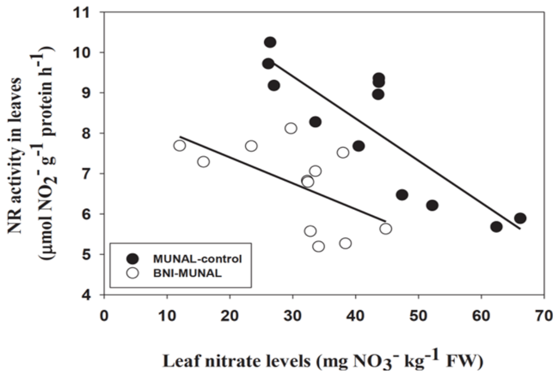
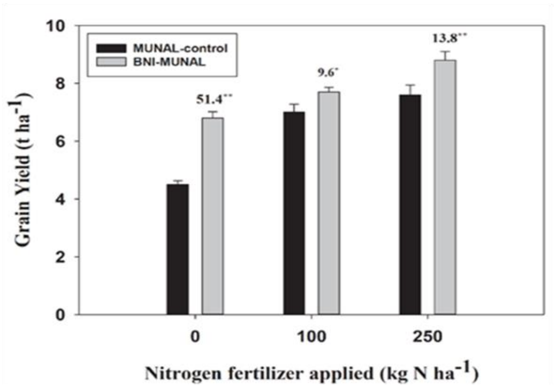


Fig. 2. N_2O emission from BNI-enabled Munal

N_2O emission was suppressed by 25%.

Fig. 3. Changes in nitrogen assimilation

BNI-enabled wheat prefers ammonium.



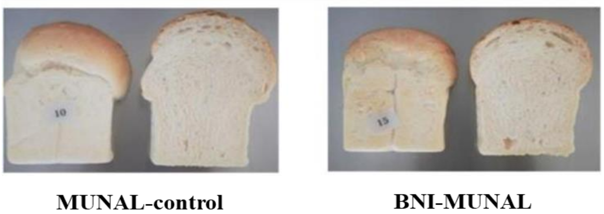


Fig. 4. Grain yield under different nitrogen application levels

No significant difference in yields between Munal-control at 250 kg/ha and BNI-enabled Munal at 100 kg/ha.

Fig. 5. Bread making quality of BNI-enabled wheat

BNI-enabled Munal can be processed into bread as well as Munal-control.

Reference: Subbarao et al. (2021) *PNAS* 118: e2106595118, https://doi.org.10.1073/pnas.2106595118