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Biological Nitrification Inhibition Enabled Wheat and its Source Chromosome

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Abstract

Wheat cultivation is one of the major causes of nitrogen pollution. Gradually accumulating soil nitrates harms human health and the ecosystem because of nitrogen leakage. This article outlines the $3Ns^b$ S chromosomal arm in wild grass (*Leymus racemosus*), which regulates the synthesis of root nitrification inhibitors and may be transferred into superior strains of wheat without compromising the elite agronomic traits. Wheat that has been enabled to exhibit biological nitrification inhibition (BNI) has the potential to enhance soil ammonium levels by decelerating its oxidation. Additionally, the assimilation of dual nitrogen forms and improved adaption to low nitrogen conditions can yield noteworthy synergistic effects. Cultivating BNI-enabled wheat on a substantial portion of the world's current wheat acreage could be an effective natural way to minimise the need for nitrogen fertiliser and minimise nitrogen losses while preserving production.

Keywords: BNI, Nitrogen use efficiency, Nitrogen leakage and Synergistic benefits

Introduction

Nitrification and denitrification are essential soil biological processes, which, left uncontrolled, can hasten the production of hazardous reactive nitrogen forms (NO_3^-, N_2O, and NO_x) setting off a “nitrogen cascade,” that harms water systems, soil fertility, and ecosystems. Modern wheat production systems are plagued by excessive nitrifier activity and a quick buildup of soil nitrates. As a result, crop nitrogen nutrition has shifted towards an "all nitrate form," which is primarily to blame for nitrogen losses in cereal crops (due to leaching in drainage water and denitrification to nitrous oxide and dinitrogen gas) and a drop in agronomic nitrogen-use efficiency (NUE), to a worldwide average of 33 per cent of nitrogen applied.

Among the three staple crops for food security, wheat utilizes around a fifth of factory-generated nitrogen fertilizer, and this percentage has not altered in the past 20 years. Reducing nitrifier usage is a practical approach for minimising nitrogen losses from agriculture. "Biological nitrification inhibition" (BNI) is the term used to describe the synthesis and dispensing of nitrification inhibitors from plant roots (mechanism illustrated in Figure 1). This increases stability and may potentially improve yield potential by streamlining the use of biochemical machinery for nitrogen assimilation. Prior research has revealed that the cultivated wheat root systems' BNI capacity is insufficiently strong to effectively inhibit soil nitrifier production in the rhizosphere.

Leymus racemosus is a perennial *Triticeae* wild grass evolutionarily related to wheat that produces larger root systems and was found to exhibit a high BNI capacity many times higher than farmed wheat due to reduced soil nitrate production and suppressed soil nitrifier activity. The chromosome Lr#n =3Ns^{b} was discovered to be mostly responsible for BNI capacity in wild grass, which was incorporated into the wheat strain, Chinese Spring, a while afterwards. This resulted in a few elite wheat varieties with a grain yield potential greater than 10 tonnes per hectare, subsequently increasing BNI capacity in root systems.

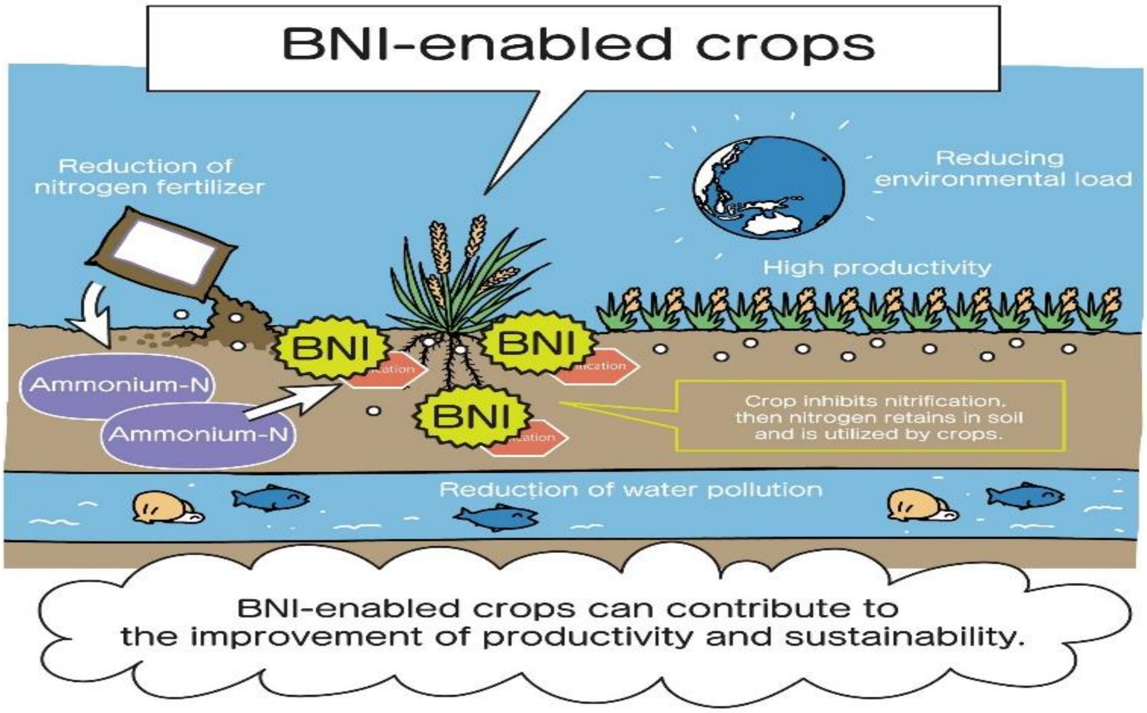

Figure 1: Functioning of BNI-enabled crops (Subbarao, G. V. *et al.* (2013)

Genetic sources for BNI Capacity in wheat and related perennial wild grasses

Among the amphiploids produced by crossing different wild wheat species and wild grasses with cultivated wheat and assessed for BNI capacity, only the *Leymus mollis Pilger* ($4\times7 = 28$, genomes $N_sN_sX_mX_m$) developed by crossing with *Triticum turgidum* showed almost seven times higher BNI capacity than Chinese Spring, as similar as observed for another wild grass species *Leymus racemosus Tzvelev* ($4\times7 = 28$, genomes $N_sN_sX_mX_m$). Both being the member of tertiary gene pool of wheat face pragmatic obstacles to the insertion of BNI genes into wheat. The majority of amphidiploid produced from wild wheat had root exudates with negative BNI activity, suggesting that they may activate nitrifying bacteria and speed up soil nitrification. Only two wild grasses, *Leymus racemosus* and *Leymus mollis* have been shown to be genetic sources thus far, for enhancing BNI capacity in wheat root systems.

Chromosomal region controlling BNI capacity in wild grasses

Leymus racemosus's high BNI activity was regulated by the short arm of chromosome Lr#n, as indicated by a twofold increase ($P < 0.001$) of BNI capacity upon transfer into Chinese Spring, contrary to long-arm translocation. The BNI trait from Lr#n is completely exhibited only in the wheat 3B translocation (T3BL.3Ns^b S), rather than the 7B translocation (T7BL.3Ns^b S). Using the ph1b mutation, three 3Ns^b S recombinant chromosomes with smaller 3Ns^b arm sizes (T3BL.3Ns^b S-Tr-3; T3BL.3Ns^b S-Tr-4; and T3BL.3Ns^b S-Tr-7) have been produced in Chinese Spring. The wheat cultivar "Chinese Spring" (CS-Lr#n-SA, commonly known as "BNI-CS") doubled in BNI capacity after T3BL.3Ns^b S was successfully introduced. Following this, T3BL.3Ns^b S from BNI-CS was introduced to a number of superior high-yielding hexaploid wheat cultivars, resulting in nearly twice as much BNI production in "BNI-MUNAL" and "BNI-ROELFS." Among all nitrogen treatments, BNI-MUNAL showed considerably greater grain yields, biomass production, and N uptake. The amounts of protein in the grain and its ability to make bread were unaffected. Broad application of BNI functions in wheat breeding may help increase adaptability to low nitrogen input marginal areas while also preventing nitrification in high nitrogen input–intensive farming.

Artificial compounds for BNI activity

Ammonia is oxidized by specific groups of microorganisms, namely, ammonia-oxidising archaea and ammonia-oxidising bacteria. Numerous substances have demonstrated the ability to reduce the activity of bacteria that oxidise ammonia. Several compounds viz., DMPP (3, 4- dimethylpyrazole phosphate), DCD (dicyandiamide), and nitrapyrin (2- chloro-6-trichloromethylpyridine) have been widely assessed for their capacity to control nitrification in agricultural systems. Their use in large-scale agriculture has been restricted because to their high cost, application problems, their short-term efficacy due to soil degradation rates that occur quickly, and, more recently, issues about the spread of DCD into food products. The in situ synthesis of nitrification inhibitors by plant roots presents a compelling and economical substitute for the use of commercial inhibitors. compounds that lessen nitrification in rhizosphere soil are released by plants; this reduces the need for laborious application procedures, ensures that inhibitors remain in surface soil where fertilisers are applied, and minimises the issue of effectiveness loss due to degradation of compounds within soil.

Process of release of biological nitrification inhibitors from roots

It was previously believed that small, uncharged molecules with low molecular weight could freely move across lipid membranes, therefore, the release of chemicals from plant roots was essentially understood to be a passive process of diffusion over the plasma membrane, for which gradient of exudates between the apoplast and cytoplasm, as well as membrane permeability, are necessary. Furthermore, it has been shown that aquaporins, also known as aquaglyceroporins, are integral membrane proteins that mediate a variety of diffusion activities by facilitating the passage of water and numerous other neutral molecules across cell membranes, including urea, ammonia, and glycerol. However, the majority of root exudates are polarised ions or electrically charged molecules, their limited solubility in the lipid membrane prevents easy diffusion across the plasma membrane bilayer. Root exudation may be a strictly controlled process as opposed to simple diffusion because in certain instances the discharge of root exudates is independent of its concentration within the root cells. Certain BNIs may be poisonous allelochemicals, implying they need to be contained in subcellular vesicles and released through exocytosis rather than existing freely in the cytoplasm. Membrane transport mechanisms such as channels, pumps, carriers, and exocytosis should therefore promote the release of BNIs from roots into the rhizosphere.

Categorization of BNI

There are two types of BNIs: hydrophilic and hydrophobic. When NH_4^+ is present in the rhizosphere with low pH, which corresponds to the activation of plasma membrane H^+-ATPase, root systems can release hydrophilic BNIs promptly. This is because plasma membrane H^+-ATPase oversees the building of membrane potential and producing a proton motive force for the secondary transport of numerous substances. The release of BNIs can most likely happen via secondary transporters, likely MATE transporters, that are driven by the proton motive force or through voltage-gated anion channels caused by fluctuations in membrane potential. Furthermore, hydrophilic BNI active efflux may also involve ATP-binding cassette (ABC) transporters. Conversely, vesicle movement and exocytosis may be involved in the release of hydrophobic BNIs from plant roots.

ROELFS-BNI and MUNAL-BNI

An economically viable, farmer-friendly, and worldwide scalable technique that encourages environmentally sound and sustainable agronomic practices is the use of BNI-isogenic lines. Since the agronomic performance and plant development of Control and BNI-isogenic lines from ROELFS and MUNAL were comparable, synthesising BNI molecules did not result in a metabolic cost. While delaying ammonium oxidation without lowering the overall population of bacteria or archaea, ROELFS-BNI and MUNAL-BNI plants reduced the abundance of ammonia-oxidizing bacteria in the soil by 60 per cent and 45 per cent, respectively. Remarkably, BNI-trait showed a synergistic impact by enabling the reduction of AOA abundance as well. In comparison to BNI-trait lacking lines, ROELFS-BNI and MUNAL-BNI plants displayed a lower leaf nitrate reductase (NR) activity due to a lower formation of soil NO_3^- and a higher amino acid content. This suggests that the transfer of Lr#-SA was able to induce a higher capacity to assimilate ammonium.

Conclusion

With largely rainfed conditions and extremely variable soil nitrogen content, the Global South accounts for over 60 per cent of the wheat area. Wheat is likely to be planted in more moderately productive locations due to the projected shortage of irrigation water. High BNI performance in low-nitrogen growing locations would become even more important when taking into account future and present hotspots for food security. The performance of the recently created BNI wheat in actual production conditions is still to be determined, especially in conditions with low to moderate nitrogen inputs. However, intensive farming with large nitrogen inputs may also result in productivity benefits owing to BNI traits. Low-nitrifying and low N_2O^- emitting production systems may be made easier with the genetic exploitation of BNI capacity in staple crop root systems. With their seed-based technology and natural solution, BNI wheats have a significant potential for increased scalability and broader use.

References

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