Nitrification Control via Crops

Hannes Karwat
Global Wheat Program (GWP)
International Maize and Wheat Improvement Center (CIMMYT), Mexico

IWYP International Wheat Yield Partnership
Research to Deliver Wheat for the Future

IWYP CONFERENCE: TRANSLATING DISCOVERY INTO IMPACT
Session 4: ROOT MICROBE INTERACTIONS AND HYBRIDS
September 10 and September 12, 2025

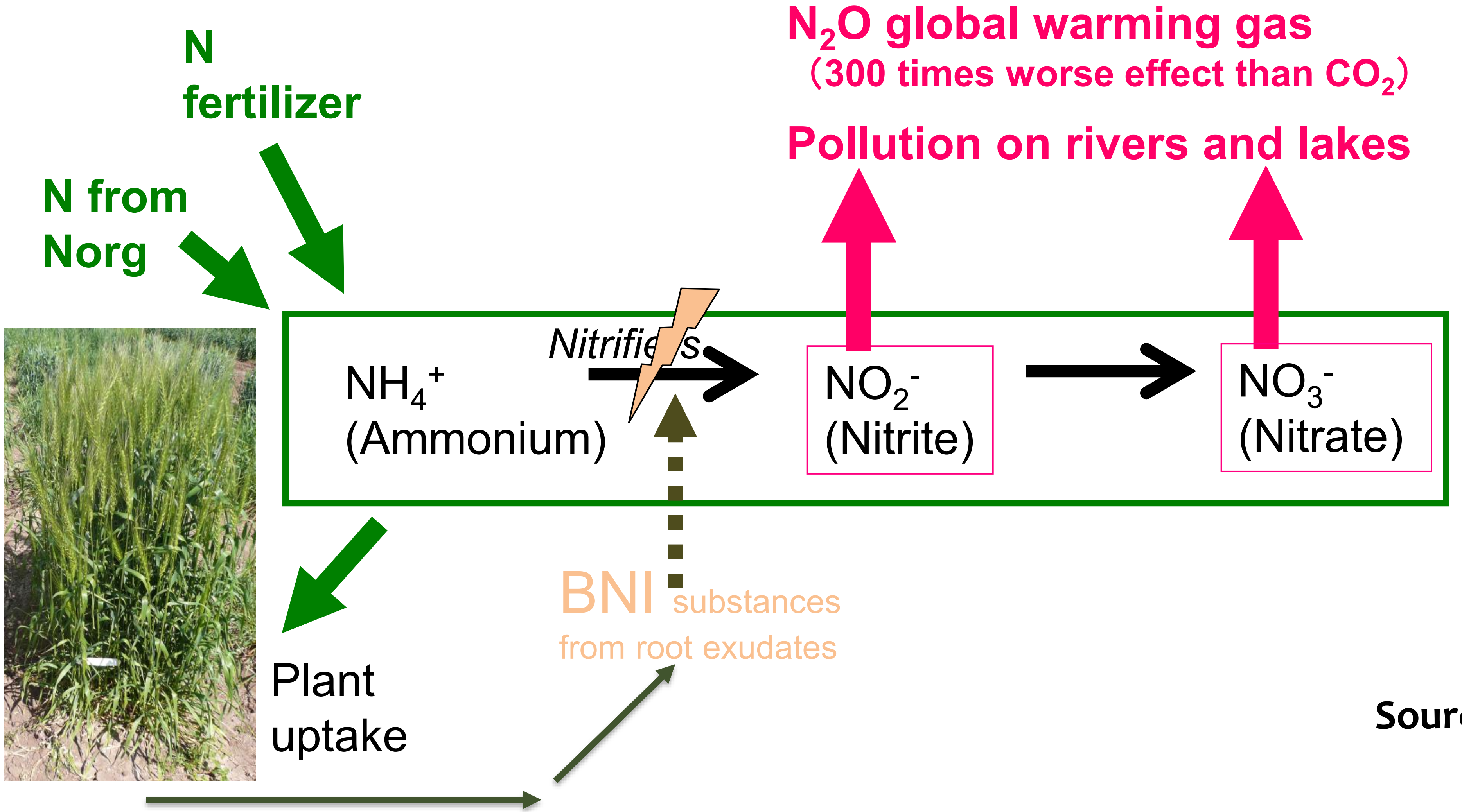
CIMMYT | CGIAR

N pollution from agriculture to the environment

- Satellite image of the Gulf of California, one month after start of crop season
- Link
- Further information:

Michael Beman, J., Kevin R. Arrigo, and Pamela A. Matson. "Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean." Nature 434, no. 7030 (2005): 211-214.

N cycle & Biological Nitrification Inhibition (BNI)



Source: Dr. M Kishii

A short history of BNI research

1983	CIAT observed *Brachiaria* pasture soil showing low nitrate level (BNI?)
1995	JIRCAS initiated collaborative research with CIAT in *Brachiaria* pasture (BNI !)
2003	Dr. Subbarao reported soil nitrification inhibition in *Brachiaria* pasture (*Plant and Soil*)
2005	1st phase, BNI research project at JIRCAS (2005-10)
2006	Introduction of the BNI concept for further crop development (*Critical Reviews in Plant Sci.*)
2009	**Confirmation of BNI in *Brachiaria* pasture** (*PNAS*) (BNI)
2010	2nd phase, BNI research project (2010-15), initiating sorghum BNI with ICRISAT
2012	**Confirmation of BNI in sorghum** (*Plant and Soil*)
2015	3rd phase, BNI research project (2015-20), initiating wheat BNI with CIMMYT
2018	Initiating **maize** BNI, and obtaining several BNI compounds (*Biology and Fertility of Soil*)
2020	Confirmation of BNI effect in **BNI-enabled wheat at field level** (*PNAS*)
2021	PNAS papers published
2022	JIRCAS introduced NNF to CIMMYT
2024	CropSustaiN “BNI Mission”

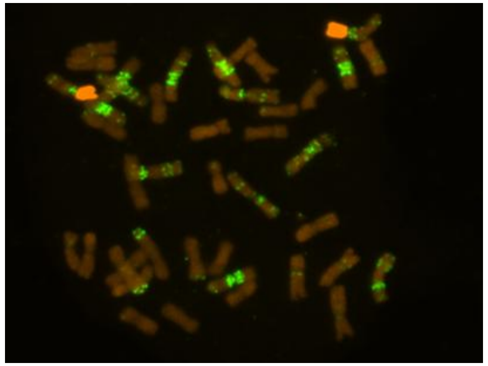
Proof of concept!

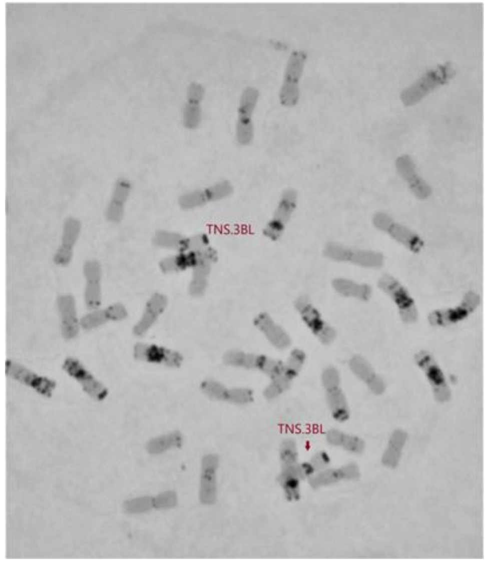
BNI capacity confirmed with bioassay (Subbarao et al. 2021)

Table 3. BNI capacity of elite wheat genetic stocks and BNI elite wheats (*SI Appendix*, Study 5a)

Serial no.	Wheat genetic stock	BNI activity released from intact plant roots (ATU · g^{-1} root dry wt. · d^{-1}) Mean	SE
1	ROELFS	86.5^{a}	12.8
2	BNI-ROELFS (CSMONO3B/3/CS/LE.RA/CS/4/CSph/5/5*ROELFS(N)	162.2^{b}	16.8
	SE of Least Square Mean ($P < 0.001$) (based on two-way analysis General Linear Model using SYSTAT 14.0)	6.99 ($P < 0.005$)	
3	MUNAL	92.7^{a}	12.1
4	BNI-MUNAL (CSMONO3B/3/CS/LE.RA/CS/4/CS/ph/5/5*MUNAL(N)	181.7^{b}	22.3
	SE of Least Square Mean ($P < 0.001$) (based on two-way analysis General Linear Model using SYSTAT 14.0)	17.9 ($P < 0.05$)	
5	NAVOJOA	91.2^{a}	22.4
6	BNI-NAVOJOA(CSMONO3B/3/CS/LE.RA/CS/4/CS/ph/4/4*NAVAJOA(N)	119.2^{a}	14.2
	SE of Least Square Mean ($P < 0.001$) (based on two-way analysis General Linear Model using SYSTAT 14.0)	22.42^{ns}	
7	QUAIU	70.2^{a}	4.9
8	BNI-QUAIU CSMONO3B/3/CS/LE.RA/CS/4/CSph/5/5*Quaiu(N)	126.4^{b}	11.8
	SE of Least Square Mean ($P < 0.001$) (based on two-way analysis General Linear Model using SYSTAT 14.0)	11.19 ($P < 0.05$)	

Holm–Sidak method—letters represent values that are significantly different ($P < 0.05$). Values are means ± SE of four replications.





BNI expression confirmed for alkaline soil & high N fertilizer application

CGIAR RESEARCH PROGRAM ON Wheat

MAFF Ministry of Agriculture, Forestry and Fisheries 農林水産省

Agriculture and Agri-Food Canada

- First observation of BNI-expression in an alkaline soil (pH 8.6-8.7) under high N fertilization (250 kg N ha^{-1})
- BNI expression in terms of lower soil nitrate in the field
- Reduced potential nitrification rates from incubation studies in soil collected one week after N application
- Indications that Lr#N SA has a secondary effect of significant higher nitrate uptake from the soil
- Yield quantity and quality stability in two of three experiments. However, harvest index of +BNI line were always lower than the HI from ROELFS
- Delayed anthesis and flowering due to the translocation were observed
- Quality analysis suggests that ROELFS-BNI BC5 needs more backcrossing

Manuscript submitted to Field Crops Research

Decision on acceptance expected within Sep 2025

Revised Manuscript (clean version) Click here to view linked References

1 **Nitrogen dynamics and yield performance of an elite bread wheat line with BNI capacity**
2 **expressed in an alkaline soil**
3 Hannes Karwat[1*], Masahiro Kishii[2], María Elena Cárdenas-Castañeda[1], Maria Itria Ibba[1], Victor
4 Kommerell[1], Alison R Bentley[3], Hans-Joachim Braun[1], Iván Ortiz-Monasterio[1]

CIMMYT | CGIAR

CropSustaiN

novo nordisk foundation

New innovative crops to reduce the nitrogen footprint from agriculture

Seeding the green transition via a natural phenomenon called BNI that works through plant root-soil interaction

Mission

1) **Verify global potential** of BNI technology to reduce the nitrogen footprint from wheat-based agriculture
2) Enable plant breeders to provide BNI-wheat seeds to farmers globally
3) Generate the 'pull' for adopting BNI technology in existing value systems from farmers, policymakers, and influencers

BNI trait is bred into wheat

Uses conventional breeding

15-20%

Potential reduction in nitrogen fertiliser usage

Depending on geography and soil nitrogen environment

CropSustaiN: Hypothesis on the table

- BNI functionality enhances N uptake & utilization of limited N by plants in **low nitrogen** environments.
- BNI mitigates N loss in **high nitrogen** environments by reducing leaching and N_2O emissions, leading to nitrogen savings.
- Environmental and field management conditions can support consistent and predictable BNI effects.
- BNI performance depends on GxExM interactions (incl. soil pH, soil structure, temperature). Such variance represents a major opportunity for breeding- and agronomy-based solutions.
- If N is used more efficiently, plants will mineralize less N, potentially leading to increased accumulation of soil C.

novo nordisk
foundation

CIMMYT

CGIAR

CropSustaiN Research Tracks

Track 1. Establish and refine a common framework that harmonizes methodologies

Track 2. Evaluate BNI trait agronomic effectiveness and stability and transfer translocations to new elite lines

Track 3. Perform field-based assessment of BNI environmental impacts

Track 4. Explore the feasibility of incorporating BNI activity into winter wheat

Track 5. Develop molecular markers associated with Leymus BNI activity

Track 6. Investigate biochemical mechanisms of BNI activity with microbial ecology

Track 7. Investigate innovation ecosystems and scaling for impact co-creation

Multi-location field trials

- **Track 2: Stability yield quality and quantity of BNI-Wheat** (**8 pairs**/12pairs*)
- N applied as ammonium sulphate in 2 split applications: **0, 75, 150** and 225* **kg N ha^{-1}**
  - **Mexico*** (El Batan, Toluca, Obregon)
  - South Asia (**India, Pakistan, Nepal**)
  - East Africa (**Kenya, Ethiopia**)
  - South America (**Uruguay**)
  - …

  = 14 trials/year
- **Track 3: N loss quantification in BNI-Wheat systems** (**2 pairs**)
  - Mexico
  - India
  - Ethiopia

  = 4-5 trials/year

Field Layout Yield Trial+: Genotype x N rate

	Border																
	150 N	8	65	3	66	5	67	2	68	4	69	1	70	7	71	6	72
REP 3	75 N	2	64	6	63	4	62	5	61	3	60	7	59	1	58	8	57
	0 N	7	49	8	50	1	51	2	52	5	53	6	54	3	55	4	56
	Border																
	150 N	1	48	6	47	5	46	4	45	7	44	3	43	8	42	2	41
REP 2	75 N	2	33	5	34	8	35	4	36	6	37	1	38	3	39	7	40
	0 N	3	32	5	31	1	30	7	29	4	28	6	27	2	26	8	25
	Border																
	150 N	1	17	3	18	8	19	5	20	6	21	7	22	4	23	2	24
REP 1	75 N	4	16	5	15	2	14	3	13	1	12	8	11	6	10	7	9
	0 N	2	1	5	2	7	3	8	4	4	5	1	6	3	7	6	8
	no plants																
	Border																

600cm

100cm

Plot width: 240cm

29.4 m

102 m

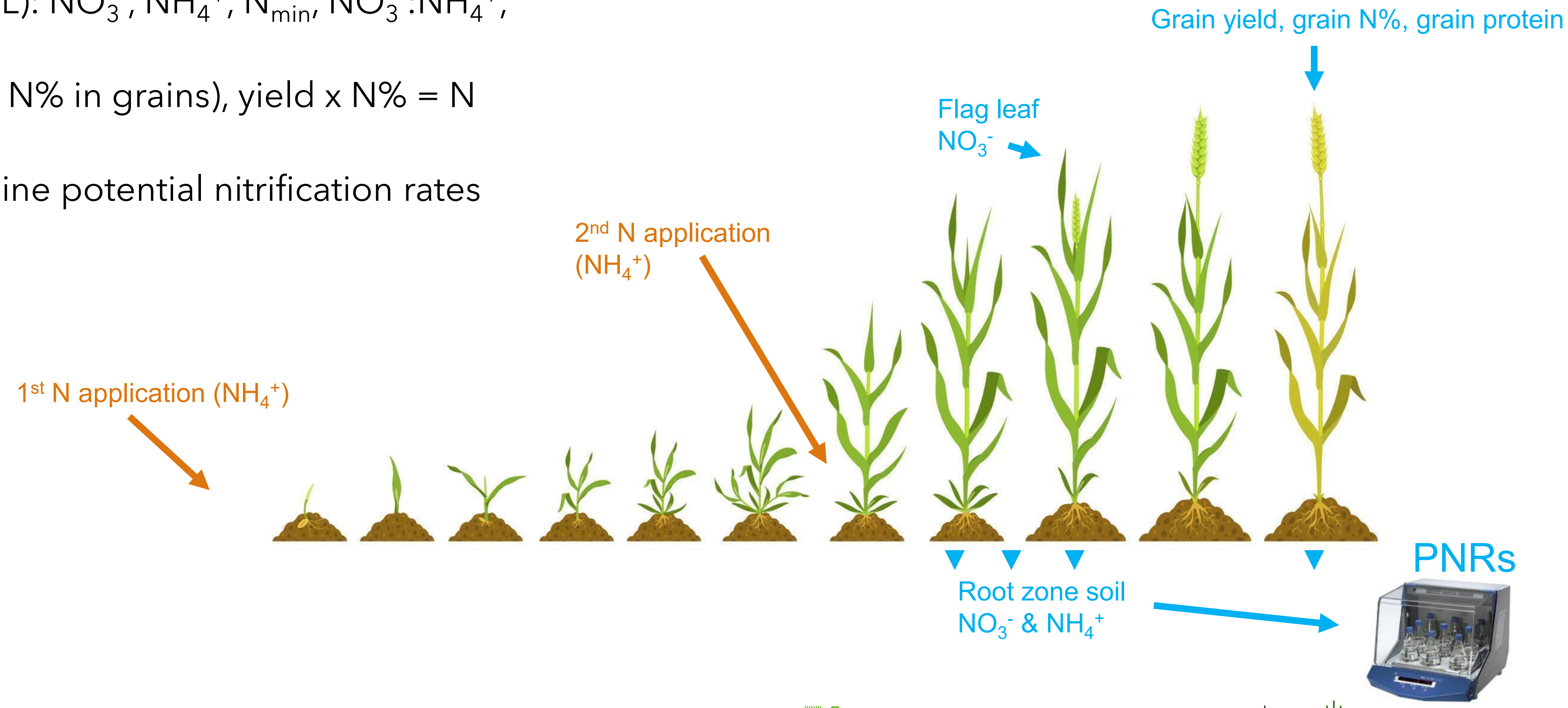
Waterchannel

GTP name

1. VOROBEY
2. VOROBEY + Lr#n-SA
3. BORLAUG
4. BORLAUG + Lr#n-SA
5. ROELFS
6. ROELFS + Lr#n-SA
7. NAVOJOA
8. NAVOJOA + Lr#n-SA

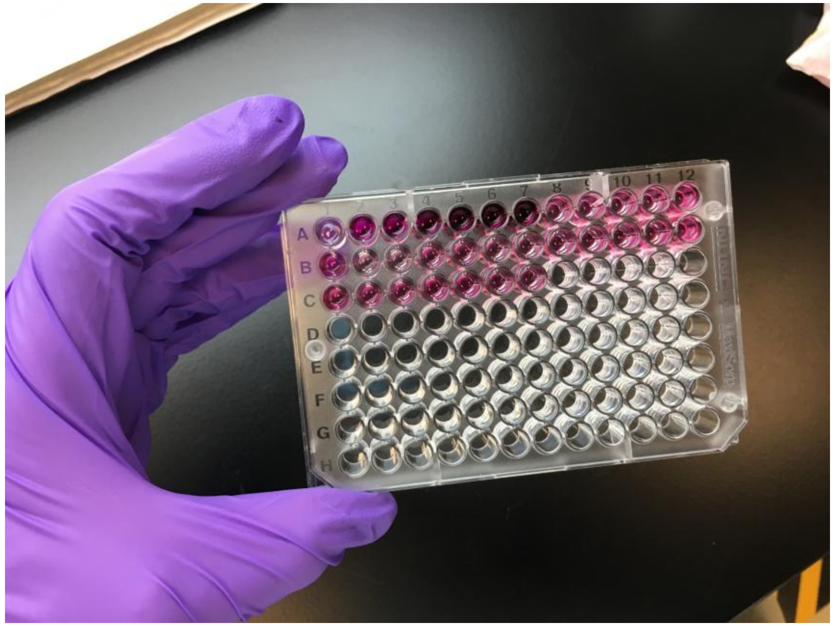
Methodology Yield Trial+

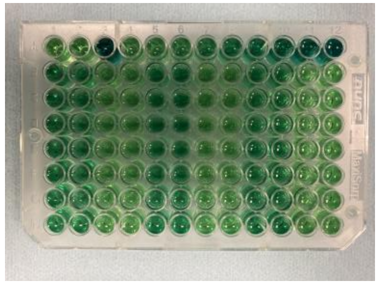
- Yield stability regardless of the introduction of the LR#N short arm to chromosome 3B
- Measurement of soil nitrate and ammonium after the second N fertilization (KCL): NO_3^-, NH_4^+, N_{min}, $NO_3^-:NH_4^+$, leaf NO_3^- (Nitrachek)
- Yield quality (protein and N% in grains), yield x N% = N uptake
- Soil incubation to determine potential nitrification rates (PNRs)

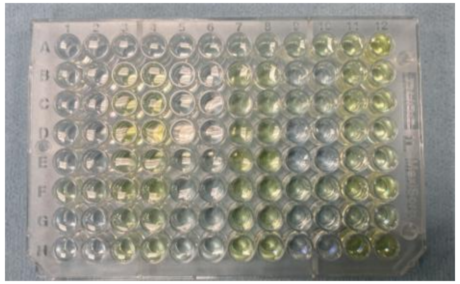


Trial establishment CropSustaiN YEAR 1

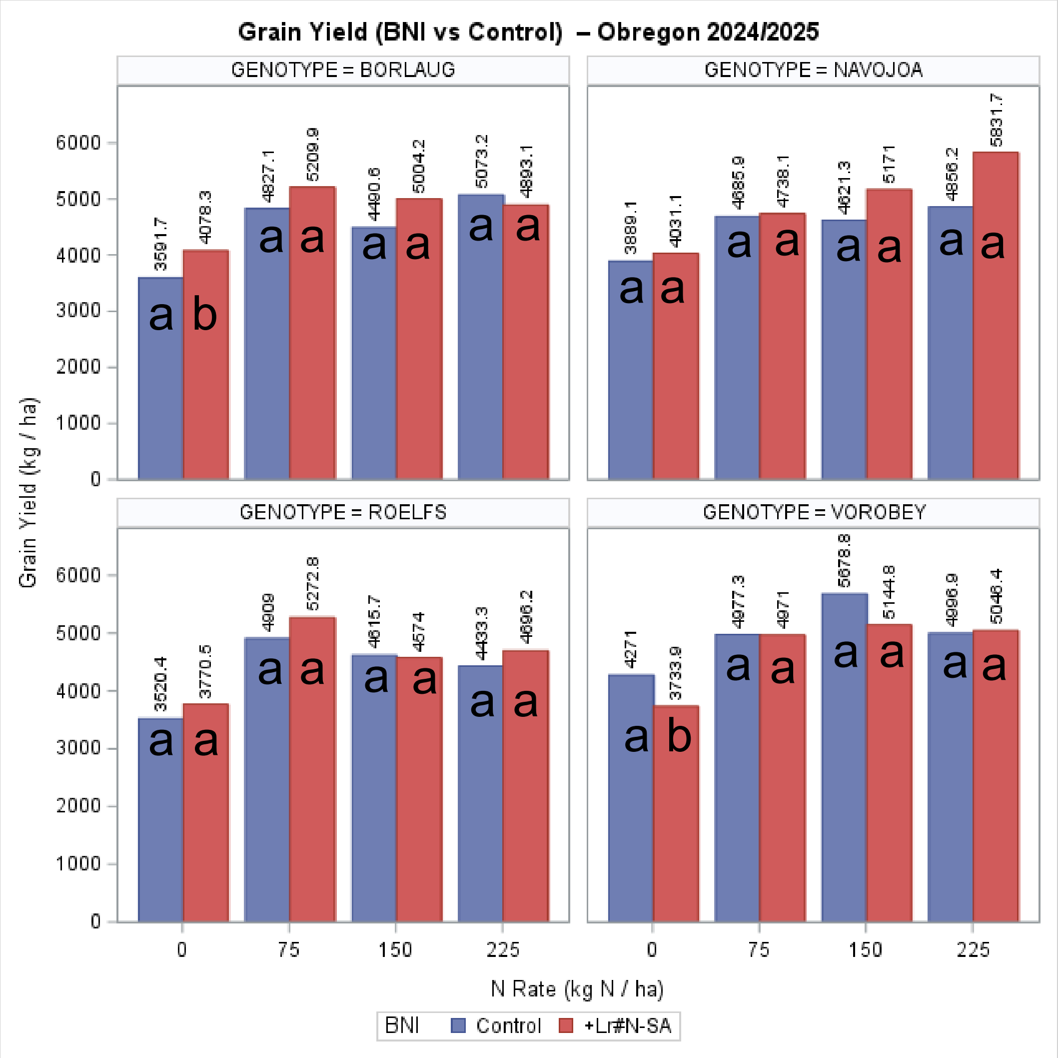
Mineral N determination during BNI/nitrification peak







Yield Trial + Obregon (MEXICO) 2024/25



BLUE = recurrent parent (control)

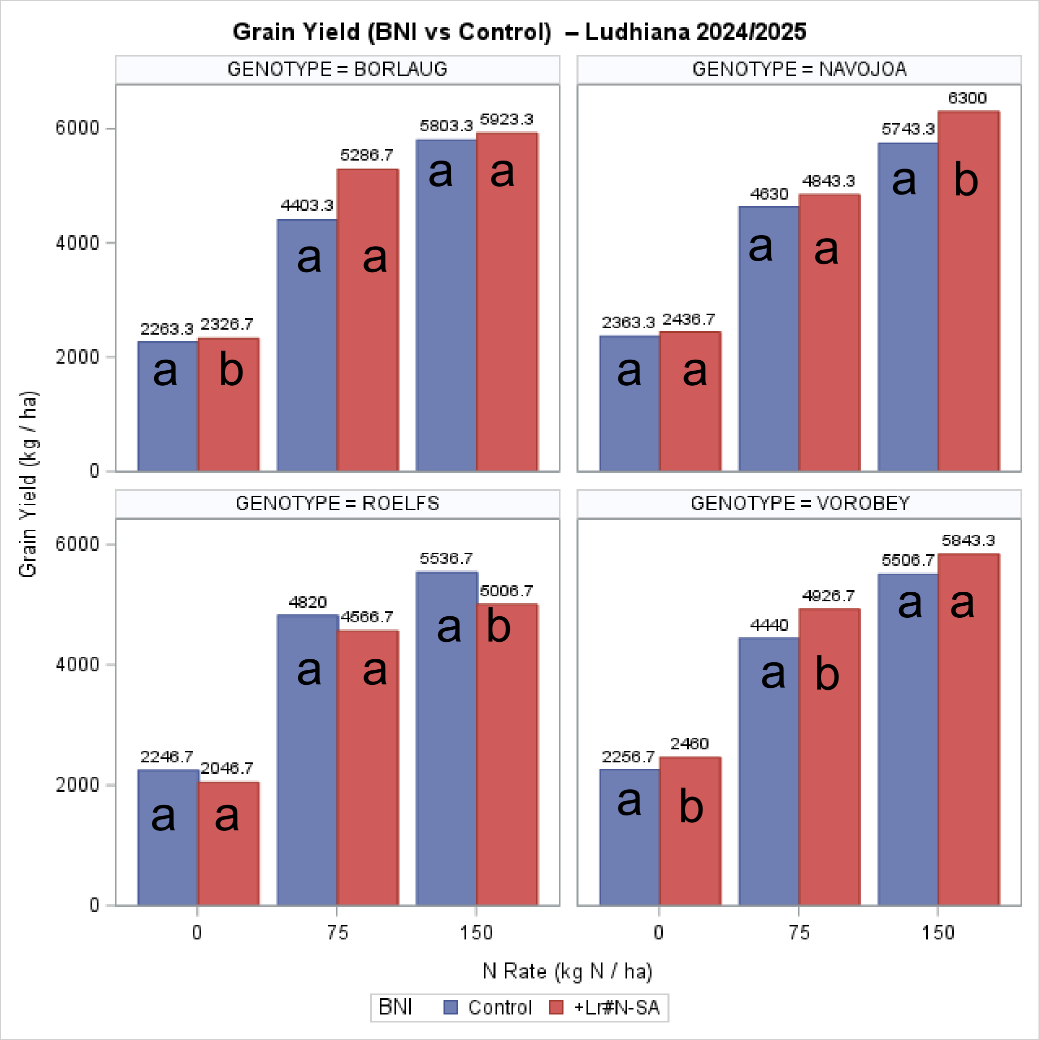
RED = BNI line

Same letters indicate no sig. difference between BNI and Control

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BNI	1	698484.17	698484.17	2.66	0.1077
N_rate	3	21274837.51	7091612.50	27.03	<.0001
GENOTYPE	3	1813987.09	604662.36	2.30	0.0852
BNI*N_rate	3	130208.60	43402.87	0.17	0.9193
BNI*GENOTYPE	3	1610417.52	536805.84	2.05	0.1163
N_rate*GENOTYPE	9	3244661.19	360517.91	1.37	0.2186
BNI*N_rate*GENOTYPE	9	1757799.32	195311.04	0.74	0.6670

- N rate strongest effect on yields
- Genotypical yield differences (p=0.0852)

Yield Trial + Ludhiana (INDIA) 2024/25



BLUE = recurrent parent (control)

RED = BNI line

Same letters indicate no sig. difference between BNI and Control

- **N rate** had the strongest effect on yield (p < 0.0001), followed by significant effects of **genotype** (p = 0.0003) and **BNI treatment** (p = 0.0052).
- The **BNI × Genotype interaction** was highly significant (p < 0.0001), showing that genotypes responded differently to the introduction of the Lr#N segment.
- Interactions between **N_rate × Genotype** (p = 0.0213) and **BNI × N rate× Genotype** (p = 0.0753) suggest genotype-specific responses to the combined effect of nitrogen levels and BNI.

Data provided by Dr. Pradeep Kumar Bhati

CIMMYT | CGIAR

Thank you for your interest!

h.karwat@cgiar.org

CIMMYT | CGIAR