

Project title:

BNI-Wheat Future: towards reducing global nitrogen use in wheat

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A research project by:

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International Maize and Wheat Improvement Center (CIMMYT), Mexico

1. Project description

Overall aim: high-precision understanding of the agronomic and N performance of BNI-Wheat, confounding and geographic factors, and development of comprehensive and globally relevant and available genetic stocks.

Overall research hypothesis: BNI-Wheat is a means to deliver improved, environmentally, and genetically stable plant and crop N use modification applicable across N levels, target geographies and genetic backgrounds for global wheat improvement.

Main activities:

- (1) To expand the testing of the existing BNI-Wheat lines in multiple field experiments reflecting different nitrogen fertilizer regimes and soil pH levels to precisely assess agronomic performance and field N balance under major known confounding factors (N input and soil pH).
- (2) To conduct multi-location international testing of existing BNI-Wheat lines at five global locations to understand the broad agronomic, N balance and product quality performance of BNI-Wheat across major target regions.
- (3) To further develop precise genetic stocks of BNI-Wheat in additional elite genetic backgrounds and multiply and distribute the material to research and downstream partners around the world.

WP1. Stability of BNI-Wheat performance at different levels of N fertilization and soil pH

Generate detailed performance and nitrogen use data on existing BNI-Wheat translocation lines, including resolution of N level and soil pH interactions. Address a current knowledge gap on the stability of BNI effects under these confounding factors to allow accurate deployment.

1.1 Using existing genetic stocks of BNI-Wheat translocations to establish multi-location field trials at three contrasting field locations (El Batan, Toluca, Obregon) in Mexico over two successive years. Each of the 12 lines will be grown in 3 replications at all three sites.

- Twelve spring wheat lines were grown and harvested in the above-mentioned locations in Mexico.
- The whole data collection for the first year is completed for all three locations.
- For El Batan and Toluca the second season (in 2024) was also completed (all samples taken, plants harvested). Post-harvest analysis (soil N analysis and seed quality analysis) is ongoing.
- The second season in Obregon is ongoing (planted in December 2024).

1.2 Manage and assess all yield trials under four nitrogen fertilizer treatments (ammonium sulphate applied in two split rates of 0, 75, 150 and 250 kg N ha⁻¹ for core agronomic traits. monitored differences of soil NO₃⁻ will be an important indicator for BNI activity. No nitrate fertilizer will be applied in any of the locations. Flowering time and height data will be collected to ensure basic agronomic suitability and trials will be harvested for quantification of yield, yield components and grain quality. N% will be measured at CIMMYT's wheat quality laboratory with near-infrared (NIR) spectroscopy in wheat grain samples to calculate agronomic NUE (N applied / grain N at harvest). Furthermore, δ¹⁵N will be measured with isotope-ratio mass spectrometry at JIRCAS, Japan.

- The trials were managed as mentioned above with an adjustment of the highest N fertilizer rate. Since the local recommendations differ among the locations, 225 kg N ha⁻¹ was chosen as high rate for all three experimental sites.
- The measurement of delta ¹⁵N natural abundance will either be done by JIRCAS in Japan after the harvest in Obregon 2025. In that case all six sets (3 locations, 2 years) will be sent to JIRCAS. Alternatively, this measurement will be conducted by another partner who has an isotope-ratio mass-spectrometer (IRMS). This collaboration could result from new networking in CropSustain.

1.3 Collect soil samples from all plots for analysis of potential nitrification rates (PNRs) before planting and at harvest, using an established soil-slurry incubation method in the soil laboratory at CIMMYT El Batan. Furthermore, mineral nitrogen (nitrate and ammonium) will be monitored (KCL extraction) in topsoil (0-10 cm) frequently after completion of N fertilizer dressing. Soil samples will be collected before the application of the second N split, and at

7, 14 and 21 days post-second N dose. Additional rhizosphere soil samples will be collected at 14 days post-second N split (peak nitrification) from trials in Obregon in Year 1 and 2 at the high N rate, lyophilized and stored for future PCR analysis of microbial communities.

- Soil samples were collected as described before planting and at harvest and during the three sampling times mentioned in Batan and Toluca in both seasons, 2023 and 2024.
- PNRs were measured in the laboratory for soil samples from planting and harvest in all three locations from the first experimental year.
- Adjustments had to be made for Obregon 2023. Soil samples could not be taken for nitrate and ammonium measurements at 7 and 14 days post-second N dose. Sampling was not possible without damaging or compacting the topsoil structure of the high-clay soil. Therefore, sampling was only conducted before planting, 21 days after the 2nd N application and at harvest.
- Soil from the planting rows (originally rhizosphere) was additionally collected from all plots from El Batan and Toluca and not, as originally planned only from Obregon. This will enable us to study the effect of the different soil pH on the nitrifier community.

1.4 Analyze existing BNI-Wheat lines using the above trialing and sampling methodology over two successive field seasons (Year 1, Year 2) to determine the agronomic, yield and nitrogen differences between 6 BNI-Wheat lines, under different N treatments and with varying soil pH conditions.

- Yield was significantly lower for BNI lines compared to control ($p=0.0005$) according to a first across-location and across all N treatments comparison.
- However, these results are influenced by a comparison of one incorrect pair. The BNI-line 'Quaiu' had been confirmed to carry the Lr-N#SA translocation, but the phenotyping clearly showed that it did not match the parental line. Furthermore, the line Sonora-64 was too low yielding for the yield comparison with the other, modern spring lines.
- The alternative comparison was the above-mentioned lines were excluded, and 4 pairs were compared across the three locations, showed no yield penalty due to the BNI-translocation ($p=0.4510$).

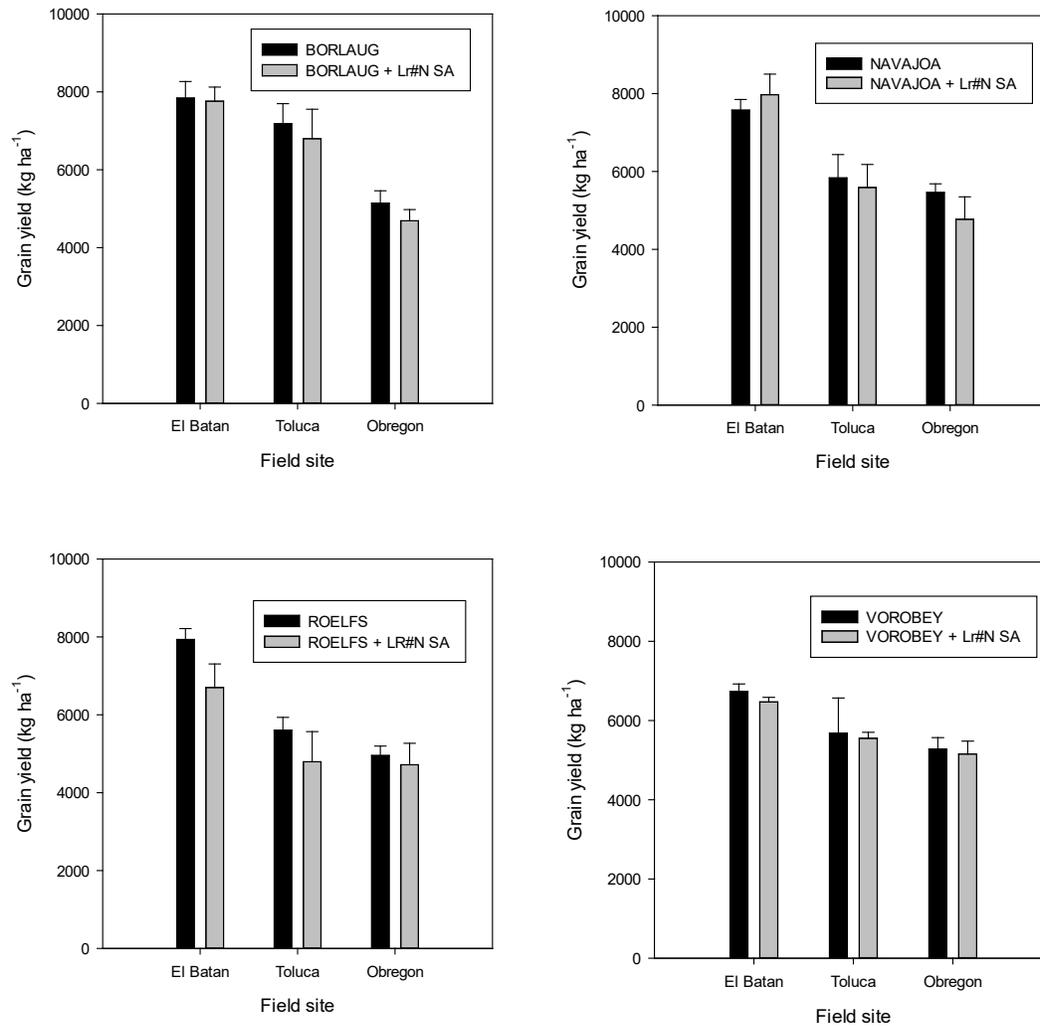


Figure1: Four out of six pairs tested in Mexico 2023 (central Mexico) and 2023/24 (Northern Mexico) are confirmed as promising experimental material. *Figures show year 1 results under full N fertilization (225 kg N ha⁻¹).* Most observations confirm yield stability of Lr#N SA translocation line compared to parental line. Roelfs + Lr#N SA had lower yields compared to parental line in two out of three field locations.

Table: Comparison of grain yield from one season in three locations and 4 iso-genetic pairs. Grain yield means are presented in kg grains per hectare.

BNI	Method	N	Mean	Std Dev	Std Err	Minimum	Maximum
NO		144	5664.0	1715.2	142.9	1361.7	8612.3
YES		144	5513.8	1662.5	138.5	1548.9	8531.8

1.5 Synthesize findings and report, including resolution of the impacts of N treatment and soil pH on BNI activity and agronomic performance in detailed field experiments.

- We compared all results from 3 different cropping season in Mexico and compared all parameters in a linear mixed model approach. Furthermore, we compared +BNI and -BNI across 4 lines, 4 N rates and 3 locations (252 N observations).
- Nitrate 3 weeks after the 2nd N fertilizer application did not differ significantly between BNI and non-BNI (p=0.2232) with estimated 11.5 mg nitrate per kg dry soil (+BNI) and 12.9 mg nitrate per kg soil (-BNI).
- Ammonium in soil at 3 weeks after the 2nd N fertilizer application was also not different between BNI and non-BNI (p=0.6603) with an estimate 18.85 ppm (-BNI) vs 18.82 ppm (+BNI).
- Nitrate-to-Ammonium ratio follows the above-mentioned results. The iso-lines did not differ among each other (p=0.6730).
- We measured both nitrate and ammonium in the planting row at harvest. With an average of over 252 observations, nitrate in soil of BNI lines were 16.25 mg per kg dry soil. The control lines had a respective nitrate concentration of 21.6 mg per kg dry soil. That is 24.7% less nitrate after the harvest.

BNI	Method	Mean	95% CL	Mean	Std Dev	95% CL	Std Dev
NO		21.5733	18.2239	24.9226	26.9967	24.8275	29.5844
YES		16.2490	14.2762	18.2218	15.9016	14.6239	17.4259
Diff (1-2)	Pooled	5.3243	1.4465	9.2020	22.1549	20.8652	23.6158
Diff (1-2)	Satterthwaite	5.3243	1.4443	9.2042			

- Ammonium in soil after harvest was almost similar. No significant statistical difference among the lines was observed (p= 0.8888).
- Nitrate-to-ammonium ratio in soil at harvest was 1.98 for BNI-lines versus 2.54 for non-BNI lines and consequently statistically significant (p= 0.0225).

BNI	Method	Mean	95% CL	Mean	Std Dev	95% CL	Std Dev
NO		2.5386	2.1337	2.9435	3.2636	3.0013	3.5764
YES		1.9784	1.7174	2.2394	2.1039	1.9349	2.3056
Diff (1-2)	Pooled	0.5602	0.0796	1.0407	2.7457	2.5858	2.9267
Diff (1-2)	Satterthwaite	0.5602	0.0794	1.0409			

WP2. Multi-environment agronomic, soil N and plant N performance in an international context

Objective: determine the overall agronomic, soil and plant N performance of existing BNI-Wheat lines in key international testing environments representative of CIMMYT spring wheat target geographies.

2.1 Multiply seed of existing BNI-Wheat lines and recurrent parents for international distribution using Mexicali field multiplication to ensure all material is pure and free of pests and pathogens to meet international plant health requirements.

- Approximately 30 kg grains from each out of 30 lines multiplied in Mexicali 2023/24 were shipped back to CIMMYT in June 2024. The seeds underwent health tests in our laboratories. Material was sent by the International Nursery to collaborators. See Annex I.

2.2 Based on Year 1 Mexico-based testing (WP1) develop a comprehensive protocol and Standard Operating Procedure for BNI-Wheat testing to provide to partners at international testing sites. This detailed protocol (SOP) will include the full description of the lines, plus the core traits to be assessed in the trials (see 2.3 for initial trait targets, these may be refined based on Year 1 WP1 trials) along with step-by-step guides on the assessment of each trait and the necessary data to be generated for analyses.

- The protocols have been developed and already shared with partners. Furthermore, these protocols will be the baseline to establish a global BNI-wheat testing networking with CropSustain.

2.3 Establish field trials of existing BNI-Wheat lines in five CIMMYT wheat target countries (South Asia: India, Pakistan, Nepal; East Africa: Kenya, Ethiopia) at two N levels (normal/mid-rate and high N). All trials will use the protocols and SOP developed and used in 2.2. Ammonium sulphate, applied and incorporated within the planting row, will be used as the N fertilizer source to ensure the highest level of precision is achieved (as in 1.2, and as will be detailed in the trial SOP developed in 2.2). The initial set of core traits to be collected in the trials are yield, yield components, grain samples for NIR analysis of grain protein and N content and calculation of agronomic NUE (as in 1.2). Given the current discussions between CIMMYT and JIRCAS to finalize the SMTA and OMTA conditions for sharing germplasm with national program partners for breeding use in the Global South, the exact locations and partner agreements will be finalized in Year 1, allowing international trialing to commence in Year 2.

- Field trials in South Asia started in the winter season 2024/25 in four locations:
 - 2 sites in India (Karnal and Ludhiana)
 - 1 site in Pakistan (Islamabad)
 - 1 site in Nepal (Bhairahawa)
- One seed-multiplication trial will be planted in January in Kenya (off-season)

- Field trials in Eastern Africa will start in the summer season 2025 in Kenya and Ethiopia. The exact locations need to be defined. The security situation for Ethiopia is critical and alternative locations in other countries (e.g. Zimbabwe) will be discussed.

2.4 Collection of soil samples post-harvest from topsoil in all plots at all sites for analysis of soil mineral N status (nitrate and ammonium). The samples will be collected from 0-10cm depth in all plots for analysis using the standard KCL extraction method (as in 1.3). This will give a snapshot value of the final N status from international trials and determine the feasibility of routine collection of soil mineral N status.

- This activity will begin in India (site 1 Karnal, site 2 Ludhiana), Pakistan and Nepal at harvest time (May 2025).

2.5 Analyze over two successive field seasons (Year 2024 & Year 2025) to determine the agronomic, yield and nitrogen differences between BNI-Wheat lines, under different field and regional conditions.

2.6 Synthesize findings and report, including resolution of the impacts of different environment x year interactions on BNI-Wheat effects on agronomic performance across key target CIMMYT geographies. Distribute trial SOP alongside future international trialing.

WP3. Consistency of performance of BNI-Wheat translocations across genetic backgrounds

Objective: continue the development of BNI-Wheat genetic resources through the completion of existing BNI translocations into elite CIMMYT wheat backgrounds and ensure sufficient high-quality seed available to research and downstream partners around the world.

3.1 Finalize the production of BNI-Wheat translocations in recent, elite CIMMYT spring wheat germplasm adapted to global production environments. All new crosses for CIMMYT Target Production Environments (TPEs) will use the newest variety releases for each TPE. This includes the transfer or new (or currently unconfirmed) translocation from both available BNI sources (*L. racemosus* and *L. mollis*) with a selection of the new or currently unconfirmed translocations.

3.2 Use cytogenetic approaches to confirm translocations present in all proposed combinations before sending them to JIRCAS for analysis of BNI activity (3.3).

- GISH-tests to confirm the translocation had been conducted for all material in WP1 and WP2. The protocol was confirmed to work very well.

3.3 Confirm the BNI activity of all new BNI-Wheat translocations in new elite backgrounds using the established protocols at JIRCAS (JIRCAS in-kind activity). BNI activity will be determined using the existing method at JIRCAS whereby plants are grown hydroponically in controlled environments to determine the expression of BNI.

- This activity will be shifted to a new partner (University of Copenhagen) in the framework of CropSustain.

3.4 Establish a new CIMMYT Special International Nursery (BNI-Wheat) to facilitate global trialing of existing and new BNI-Wheat lines. Complete Mexicali seed multiplication (to ensure global seed health requirements are met) of existing material (Table 1) and commence multiplication of new lines (Table 3) for further testing and distribution. In total, four rounds of seed multiplication within the project to multiply and ensure purity and availability of BNI-Wheat genetic stocks.

- During the first winter season (2023/24) 30 entries were multiplied in Mexicali. In 2024/25 (ongoing) 40 entries are multiplied in Mexicali to receive disease-free high-quality seeds for distribution to partners. Around 30 kg of seed material from each entry was and will be sent after harvest from Mexicali back to CIMMYT headquarters in El Batán.

Table1: List of germplasm currently multiplied in Mexicali 2024/2025.

# ORDEN	GERMPLASM NAME	GERMPLASM CODE	PACKAGE LABEL
1	CA18B00104M-5B-0T3B	WGE000011627453	GHB2022\C.MOLECULAR\1
2	CA18B00098M-5B-0T3B	WGE000011627454	GHB2022\C.MOLECULAR\2
3	-29B-0T3B	WGE000011627455	GHB2022\C.MOLECULAR\3
4	CA18GHB01220M-9B-0T3B	WGE000011627456	GHB2022\C.MOLECULAR\4
5	CA18GHB01081S-5B-0T3B	WGE000011627459	GHB2022\C.MOLECULAR\7
6	CA18GHB01225M-5B-0T3B	WGE000011627460	GHB2022\C.MOLECULAR\8
7	CMSS97Y04045S-040Y-050M-040SY-030M-14SY	WGE000004313703	GHB2022\C.MOLECULAR\10
8	CMSS96Y02555S	WGE000000297372	GHB2022\C.MOLECULAR\11
9	CGSS00B00169T-099TOPY-099M-099Y-099M-9	WGE000004905617	GHB2022\C.MOLECULAR\12
10	CMSS06B00734T-099TOPY-099ZTM-099Y-099M	WGE000006175063	BW21B-002-0001
11	CMSS06Y00605T-099TOPM-099Y-099ZTM-099Y	WGE000007806808	GHB2022\C.MOLECULAR\14
12	CA19GHB00466M-17B-0T3B	WGE000011627462	GHB2022\C.MOLECULAR\16
13	CA19GHB00466M-18B-0T3B	WGE000011627463	GHB2022\C.MOLECULAR\17
14	CA19GHB00493M-9B-0T3B	WGE000011627464	GHB2022\C.MOLECULAR\18
15	CGSS01B00046T-099Y-099M-099M-099Y-099M	WGE000005398757	GHB2022\C.MOLECULAR\20
16	CA21GHB00595M-0GHB-1T3B-1GHB-0Y	WGE000009734514	Y22-23\L.TRANS 3B\4
17	CA21GHB00595M-0GHB-2T3B-1GHB-0Y	WGE000009734515	Y22-23\L.TRANS 3B\5
18	CA20GHB00879M-0GHB-1T3B-1GHB-0Y	WGE000009734516	Y22-23\L.TRANS 3B\6
19	CA20GHB00882M-0GHB-1T3B-1GHB-0Y	WGE000009734517	Y22-23\L.TRANS 3B\7
20	CA19GHB00493M-10B-0T3B	WGE000011627465	GHB2022\C.MOLECULAR\19
21	CA21GHB00395M-0GHB-1T3B-1GHB-0BGH	WGE000012180820	WC22B-003-0023
22	CA18B00104M-14B-0Y	WGE000012178374	WC22B-001-0026
23	CA21GHB00436M-0GHB-1T3B-1GHB-0Y	WGE000009734875	Y22-23\L. 2TRANS\24
24	CA20GHB00059M-0GHB-34T5B-1GHB-0BGH	WGE000012180869	WC22B-002-0002
25	CMSS11B00123S-099M-099NJ-099NJ-2RGY-0B	WGE000007955575	MXI21-22\MRUSTCHECKS\1
26	CA21GHB00619M-0GHB-3T3B-0BGH		
27	CA20B00257S-0GHB-1TMol-2GHB-1Y-0BGH	WGE000012180797	WC23A-022-0001
28	CASS22GHB00017T-1GHB		
29	CA18B00104M-15B-1Y-1B-0Y	WGE000012274942	WC23B-015-0003
30	CA18B00104M-18B-1Y-1B-0Y	WGE000012274943	WC23B-015-0004
31	CA18B00104M-18B-2Y-1B-0Y	WGE000012274944	WC23B-015-0005
32	CA21GHB00395M-0GHB-1T3B-0GHB	WGE000012186015	WC23A-023-0024
33	CA21GHB00443M-0GHB-1T3B-1GHB-1Y-0B	WGE000012176114	WC23B-010-0012
34	CA21GHB00443M-0GHB-1T3B-1GHB-2Y-0B	WGE000012176115	WC23B-010-0013
35	CA21GHB00443M-0GHB-1T3B-1GHB-5Y-0B	WGE000012176118	WC23B-010-0016
36	CA21GHB00828M-0GHB-1GHB-1GHB-4Y-0B	WGE000012176157	WC23B-013-0001
37	CA21GHB00828M-0GHB-1GHB-1GHB-2Y-0B	WGE000012176155	WC23B-013-0008
38	CA21GHB00828M-0GHB-1GHB-1GHB-5Y-0B	WGE000012176158	WC23B-013-0002
39	CA21GHB00828M-0GHB-1GHB-1GHB-1Y-0B	WGE000012176154	WC23B-013-0007
40	CA21GHB00828M-0GHB-1GHB-1GHB-3Y-0B	WGE000012176156	WC23B-013-0009

3.5 Report on development of new germplasm and availability of seed stocks. Distributed seed to partners based on requests received.

Annex I. [MasterFile Seed Distribution.xlsx](#)



MasterFile Seed
Distribution.xlsx