Australian Government
Australian Centre for
International Agricultural Research

# Final report

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| *Project full title* | **Identification of sources of resistance to wheat blast and their deployment in wheat varieties adapted to Bangladesh** |
| *project ID* | CIM/2016/219 |
| *date published* | 23/02/2022 |
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| *approved by* | Dr Eric Huttner |
| *final report number* | FR2021-081 |
| *ISBN* | 978-1-922787-04-0 |
| *published by* | ACIAR<br>GPO Box 1571<br>Canberra ACT 2601<br>Australia |

# Contents

# 1. Acknowledgments

The authors are grateful to the partners in India, China, Pakistan, and Nepal for providing wheat germplasm for WB screening, to the partners in ARI of USA, Japan, Australia, and Sweden for their scientific contribution, and to partners and technical staff in Bolivia, Bangladesh, and CIMMYT for their contribution in screening against WB, breeding, laboratory analysis, and seed multiplication and shipment. Special thanks to the administrative staff of ACIAR for their support on the implementation of this project.

# 2. Executive summary

Wheat blast (WB) is a serious constraint to wheat production in the warmer areas of South America. It has now spread to Bangladesh in 2016 and Zambia in 2018, posing great threat to the wheat production in Asia and Africa, respectively, and having a potential to become a globally important wheat disease. Wheat is the second largest cereal consumed in Bangladesh and the threat of WB hinders the government's ambition to increase domestic wheat production. The aim of this project was to identify sources of resistance, understand the genetics of resistance and breed WB resistant varieties adapted to Bangladesh. With the synergic efforts of CIMMYT, the Bangladesh Wheat and Maize Research Institute, and the National Institute of Agricultural & Forestry Innovation of Bolivia, three WB screening platforms, one in Bangladesh and two in Bolivia, have been established for high-throughput screening of wheat materials for WB resistance. During this project, nearly 10,000 wheat genotypes from CIMMYT, South Asia, and China have been evaluated in the three field screening platforms for WB over multiple cropping seasons. Based on the WB data, resistant sources have been identified for direct release or resistance breeding in WB-prone areas. Genetic studies have also been conducted to identify QTL for WB resistance and their flanking markers for potential utilization in marker assisted selection. The results indicated, however, that resistant sources in the tested genotypes have mostly been limited to lines carrying a translocation on chromosome 2A containing an alien chromosome 2N segment from *Aegilops ventricosa,* termed the 2NS/2AS translocation. In this report, the translocation is referred to simply as "2NS", and the lines containing it are called "2NS lines". The non-2NS genotypes were mostly susceptible to WB, with only around 20 genotypes exhibiting resistant or moderately resistant reaction across experiments, having potentiality to be used as alternative WB resistant sources for breeding. Similarly, genetic studies have all indicated that the 2NS/2AS translocation region being the only stably expressed QTL with major effects across experiments. Additional QTL have also been identified in non-2NS regions, but their phenotypic effects were all less than 10%, with their expression varied greatly across experiments. Regarding the WB resistance breeding, more than 100 crosses have been made in 2017 in CIMMYT and Bangladesh, and the progenies have been incorporated into the respective wheat breeding pipelines for future varietal release. In the last few years, five, two, two and one WB resistant varieties have been released in India, Bangladesh, Bolivia, and Nepal, respectively, owing to the WB screening work conducted in this project. Around 100 scientists from Bangladesh and countries at risk of WB have been trained over the course of the project. Future activities will include the ongoing search for non-2NS resistant sources, the identification and mapping of effective resistance genes, the fine mapping of the 2NS resistance gene, the pyramiding of multiple sources of WB resistance, and the breeding and dissemination of high-performing WB-resistant wheat varieties adapted to the various wheat growing areas of Bangladesh.

# 3. Background

Wheat blast (WB) is a devastating fungal disease threatening the wheat production in the tropical and subtropical regions. The causal agent is *Magnaporthe oryzae* pathotype *Triticum* (MoT), a pathotype closely related to the rice blast pathogen *M. oryzae* pathotype *Oryza* (MoO). WB is a relatively new disease in wheat, being first reported in the Parana State, Brazil in 1985 (Igarashi et al., 1986) and soon spread to the main wheat growing regions in Brazil, as well as to the neighbouring countries Bolivia, Paraguay, and Argentina (Kohli et al., 2011). In South America, this disease is among the major biotic constraints to wheat production, affecting up to 3 million hectares. Yield reductions are often significant and can lead to 100% crop loss under conducive conditions for the disease (Kohli et al., 2011; Duveiller et al., 2016). Disease development of WB is very rapid, and the spikes of susceptible varieties normally become completely withered within only a few days after the first appearance of symptoms, giving limited time for farmers to deploy remedial measures. Farmers in WB prone areas often make preventative fungicide applications of two to three times around anthesis, regardless of the presence of the WB symptom, yet severe yield losses happen. A good example happened in 2005 in Brazil, when two fungicidal applications were made against WB, but yield reductions of 14 to 32% were recorded (Urashima et al., 2009), demonstrating the limited effects of pre-emptive fungicide application.

Although WB had long been confined to South America, prediction models based on weather parameters have identified risk regions for WB epidemics in the tropical and sub-tropical regions of India, Bangladesh, Pakistan, Ethiopia, China, USA, Australia (Cao et al., 2011; Cruz et al., 2016a; Duveiller et al., 2016). In February 2016, the prediction came true in Bangladesh, where the first WB epidemic outside South America was recorded. Characterization of the Bangladeshi WB isolates demonstrated their high genetic similarity to isolates from South America (Islam et al., 2016; Malaker et al., 2016), in accordance with the wheat trade history from South America to Bangladesh (Ceresini et al., 2018). In subsequent years, WB disease continued to spread to new districts in Bangladesh despite the unfavourable weather conditions, demonstrating its adaptation to local conditions and its eradication to be almost impossible (Islam et al., 2020; Mustarin et al., 2021; Singh et al., 2021). The introduction of WB into Bangladesh has caused great concern for food security in the South Asia region, considering the high consumption of wheat of more than 100 million tons annually. More importantly, some tropical and sub-tropical areas of India, Nepal and Pakistan have similar climatic conditions to Bangladesh, implying a potential danger of WB epidemics in the broader region. In 2018, WB was identified for the first time in Zambia (Tembo et al., 2020) due to the importation of South American grain, too, and the epidemic region in the country has also been expanding in recent years (Singh et al., 2021). It is very likely that WB keeps spreading globally considering 1) the MoT pathogen can be disseminated along the contaminated grains through global wheat trade, 2) the changing climate (global warming and irregular rains) and the fast evolution of the MoT pathogen (increased virulence, fungicide resistance and sexual recombination) could further drive local or regional disease expansion, and 3) the host jump mechanism might result in the *in-situ* emergence of MoT. Host jump has occurred in South America (Inoue et al., 2017) and could happen in other regions having *M. oryzae* pathotypes closely related to MoT, like USA (Farman et al., 2017), China (Wang et al., 2021), and Australia (Pak et al., 2021).

Host resistance to WB is regarded as the most effective, economical, and environment-friendly approach to manage the disease. This is especially true for developing countries like Bangladesh, where wheat producers are mostly resource-poor small-scale farmers. South American researchers and breeders have evaluated diverse germplasm ever since the first WB epidemic in 1985 to identify resistant or tolerant sources; but these activities relied heavily on natural WB infection, which happened sporadically, making the progress of WB resistance breeding very slow. The 2016 WB outbreak in Bangladesh ignited a series of projects to identify genetic resistance, including the current project, which have led to the release of several WB resistant varieties in Bangladesh, India, Nepal, and Bolivia: e.g., BARI Gom 33 in Bangladesh (named 'INIAF Okinawa' when released in Bolivia), WMRI Gom 3 in Bangladesh (named 'INIAF Tropical' when released in Bolivia and Borlaug 2020 in Nepal) and DBW187, HD3249, DBW252 and HD3293 in India.

Despite the progress, it has been found that almost all the resistant genotypes identified so far contain the same resistance locus that resides on the 2NS chromosomal segment introduced from *Aegilops ventricosa* (Zhuk.) (He et al., 2020; Juliana et al., 2019; Singh et al., 2021). This chromosomal segment confers resistance to multiple biotic stresses, e.g., rusts (Helguera et al., 2003), cereal cyst nematode (Jahier et al., 2001) and root-knot nematodes (Williamson et al., 2013). Cruz et al. (2016b) reported that 2NS reduced WB severity by 64-81% in both spring and winter wheat, although they also demonstrated lower levels of resistance conferred by 2NS against some recent MoT isolates. This has been recently corroborated by Cruppe et al. (2020), clearly showing that the resistance conferred by 2NS is being gradually eroded. It has been well known in the resistance breeding work on rusts and powdery mildew that relying on single resistance genes is risky, because virulent strains can evolve rapidly to overcome the 'resistant' varieties. Identification of non-2NS based resistant genotypes for wheat production in WB prone regions would reduce such risk. So far, only a few non-2NS resistant sources have been reported (Cruppe et al., 2020; He et al., 2021; Roy et al., 2021b), and more efforts are needed to obtain new sources of resistance through screening of larger panels of previously uncharacterized germplasm.

Immediately after the 2016 WB outbreak in Bangladesh, a policy called 'Wheat Holiday' was implemented in both Bangladesh (Mottaleb et al., 2019a) and the bordering districts of West Bengal, India, to prevent the disease spread (Mottaleb et al., 2019b). Nevertheless, the disease kept spreading in Bangladesh in the subsequent years despite the unfavourable weather conditions, which implies that WB is now endemic in the country. At the same time, the Bangladesh government is striving to significantly increase wheat production, thus the 'Wheat Holiday' policy has been suspended, and active management strategies have been adopted in the country (Islam et al., 2019). Apart from the cultivation of WB resistant varieties mentioned above, management of this disease is composed mainly of fungicide application and cultural approaches.

Fungicide application is an important method to manage WB, yet its effects vary greatly under different disease pressure and active chemical components. Additional challenges include the evidenced risk of fungicide resistance in new MoT isolates (Castroagudín et al., 2015), limited access of small-holder farmers to fungicides, and the negative impacts of fungicides on environment and the cost of production. Nevertheless, fungicides remain an effective remedial method to reduce yield loss when other management methods are not available, and a few fungicides released in Bangladesh have shown effectiveness against WB, for example Nativo 75 WG (Roy et al., 2020).

Several cultural approaches like sowing date adjustment (BWMRI, 2020), crop residues removal, and deep tillage have shown effectiveness in WB management in South America and Bangladesh. Except for sowing date adjustment, these methods are counter to recommended conservation agricultural strategies. In Bangladesh, late sowing is associated with hot and humid weather conditions favourable for WB, spot blotch, and terminal heat, and thus timely sowing in the second half of November is important to avoid severe WB infection (He et al., 2020).

Because no WB immunity has been found in wheat germplasm, other management strategies, especially fungicides and timely sowing, are indispensable for the WB management package in Bangladesh, which, together with WB resistant or tolerant varieties, contribute to the WB management in the country.

# 4. Objectives

1. Identify sources of WB resistance that can be utilized by breeders, pathologists, and geneticists for germplasm characterization and enhancement;

2. Determine the genetics of WB resistance and identify molecular markers linked to resistance. Develop the molecular tools needed for rapid transfer of WB resistance into elite germplasm;

3. Develop agronomically superior WB resistant lines with other traits critical to Bangladesh (heat tolerance, early maturity, resistance to spot blotch and leaf rust);

4. Evaluate and release improved varieties, including the increase and dissemination of quality seed.

# 5. Methodology

## 5.1 Wheat blast screening

**Precision phenotyping platforms (PPPs).** Development of PPPs for WB in Bangladesh and Bolivia was a prerequisite for WB screening and the subsequent genetic and breeding activities. In Bangladesh, the PPP was set up in a lowland south-western city Jashore, where BARI's regional research station is located. The wheat cycle runs from December to April, having similar sowing and harvest dates for much of Southern Bangladesh where WB has been epidemic. In Bolivia, the PPPs were located at two WB endemic locations, Quirusillas (highlands, wheat crop cycle from December to March) and Okinawa (lowlands, wheat cycle from May to September), providing two cycles of disease evaluations per year. Two sowings separated by two weeks were made in each location to expose the materials to wider climatic conditions. All the PPPs were equipped with a mist irrigation system to provide a WB conducive environment. Field inoculation was done with spraying a mixture of locally collected high-aggressive MoT isolates once at the flowering stage and the second at two days after first inoculation, using a backpack sprayer. Disease evaluation took place two or three weeks after the first inoculation, depending on the WB progress, and WB index was used to represent both WB incidence and severity. Important phenological traits like days to heading (DH) and plant height (PH) were also measured. In this report, the experiments were named as per the location ('Quir' for Quirusillas, 'Oki' for Okinawa, and 'Jash' for Jashore), cropping season ('18' for the 2017-18 or 2018 cycle, and '19' for the 2018-19 or 2019 cycle etc.), and sowing ('a' for the first sowing and 'b' for the second). For example, Quir18b represents the second sown experiment in the 2017-18 cycle conducted in Quirusillas. Empirically, the tested genotypes were classified as resistant (with WB index less than 10%), moderately resistant (between 10 and 30%), moderately susceptible or tolerant (between 30% and 60%), and susceptible (greater than 60%).

The PPPs were part of a global network of Precision Field-Based Phenotyping Platforms developed by CGIAR Research Program (CRP) on WHEAT with co-investing National Agricultural Research System (NARS, in Bangladesh, the Bangladesh Wheat and Maize Research Institute BWMRI), with an objective of generating high quality phenotypic data, to accelerate varietal development and to maximize the potential of new genotyping technologies. Each PPP acts as a hub for generating and sharing data and knowledge on particular traits, as well as contributing to building good protocols, defining research agendas, promoting capacity development activities, and strengthening linkages between national and international research programs.

NARS showing interest in participating in the PPPs entered a Memorandum of Understanding with CRP WHEAT. One of the pre-conditions of establishing a PPP was to accept that there was no restriction imposed on imported wheat seed as far as it came previously certified by the competent authority of the country of origin. Seed export requirements included a phytosanitary import authorization from destination country and packing list. The country used the Standard Material Transfer Agreement and phenotyping data from wheat trials can be shared. The agreement contemplated a co-investing budget, with NARS being responsible for the salary of the scientist leading activities at the platform, field assistants, administrative costs, and facilities. In parallel, inputs and supplies related to the field operations, associated expenses, and temporary and research associates for field activities were mainly contributed by CRP WHEAT. Technical and scientific help for all operations was provided through CRP WHEAT.

**Released varieties and elite breeding materials** are the main component of accessions screened in the PPPs. The former was mainly from South Asian countries, e.g., India, Bangladesh, Pakistan and Nepal, with additional germplasm from China. It is beneficial to both Bangladesh and Bolivia, where the PPPs are located, to select locally adapted WB resistant or tolerant varieties for direct release, waiving the long breeding cycles. Moreover, the diverse resistant lines are the most important building blocks for WB resistance.

The elite breeding materials were mainly from CIMMYT international nurseries, which include new high yielding lines. The below nurseries were the main components:

- International Bread Wheat Screening Nursery (IBWSN),
- Semi-Arid Wheat Screening Nursery (SAWSN),
- Helminthosporium Leaf Blight Screening Nursery (HLBSN, spot blotch resistant germplasm),
- Harvest Plus Advanced Nursery (HPAN, high zinc content),
- Wheat Blast Elite Germplasm (WBEG),
- International Durum Screening Nursery (IDSN).

Majority of these nurseries were genotyped under the project “Rapid Development of Climate Resilient Wheat Varieties for South Asia Using Genomic Selection” (USAID Funded and led by KSU, USA) to enable subsequent genetic studies. Accessions of the nurseries possess traits necessary for varietal adaptation in Bangladesh and other areas vulnerable to wheat blast.

**Landrace, synthetic wheat lines, and wide-cross materials** accounted for a smaller proportion of the materials evaluated in the PPPs, mainly due to their phenotypic characters like very late maturity and high stature that made WB evaluation very difficult. Some landrace accessions from Mexico and Iran were evaluated, and primary and derivative synthetic wheat lines from CIMMYT, along with a few wide-cross materials were also screened. The promising materials from such nurseries were intended for pre-breeding for the introgression of WB resistance into improved germplasm for varietal development. Most of this germplasm were genotyped under the project “Seeds of Discovery”, funded by Mexico Government through its agriculture secretariat, SAGARPA, enabling further genetic studies.

## 5.2 Genetics of wheat blast resistance

This is an important work component to uncover the genetic basis for WB resistance. Due to the major effects of the 2NS/2AS translocation, most materials screened in the PPPs were tested for the translocation to inform further genetic studies. The 2NS markers utilized were STS markers Ventriup-LN2, cslVrgal3, WGGB156 and WGGB159, that have been shown to be linked to the WB QTL on the 2NS/2AS translocation region. Special attention was paid to resistant or moderately resistant genotypes without the 2NS translocation, which implied novel resistance loci. In that case, crosses were made using the identified non-2NS source with WB susceptible parents having similar phenological traits to the former to reduce the confounding effects from DH, PH etc. The bi-parental populations were generated through the single seed descent (SSD) method to advance until F7 for field experiments.

The nurseries with genotypic data available formed populations for Genome Wide Association Study (GWAS), and software packages TASSEL, GAPIT etc. were used to analyse population structure, linkage disequilibrium, and to detect genomic regions conferring WB resistance, as well as the closely linked molecular markers. Normally many Marker-Trait Associations (MTA) were detected in single environments; but only the repeatable ones, preferably those being significant across many environments, were taken as significant MTAs.

Before the initiation of this project, 10 bi-parental populations had been developed to decipher a few WB resistant sources, like Milan, Caninde#1 and Caninde#2. Those 10 populations were evaluated in the three PPPs during this project, and three of them were genotyped with DArTSeq markers at CIMMYT-Mexico for QTL mapping with ICIMapping or MapQTL programs. Similar to the GWAS panels, only repeatable QTL were regarded as significant. Additionally, QTL mapping for DH and PH were also made to check if any WB QTL coincided with DH or PH QTL, with an aim to eliminate confounding QTL.

The significant markers in the 2NS/2AS translocation region were extracted from the genotyping dataset for a haplotype analysis to identify possible recombinants, which are useful for subsequent precision mapping work of the 2NS QTL for WB resistance. A few mapping populations and nurseries were not genotyped with genome-wide markers, but genotypic data of the four STS markers mentioned above were made available, thus possible recombinants among the four markers could also be identified for future research. Once selected, those recombinants will be screened with more markers in the 2NS/2AS translocation segment, to refine the QTL region and identify more closely linked molecular markers useful for marker assisted selection (MAS). This will be conducted in the phase II of the project.

The 2NS marker genotyping was done at the wheat pathology lab. of CIMMYT, and the GWAS and QTL mapping studies were conducted synergically by CIMMYT and NARS researchers.

### 5.3 Breeding for wheat blast resistance

The general strategy for WB resistance breeding was to integrate traditional breeding, pathology, and molecular techniques to develop superior parental materials via hybridization and selection. The key components included:

- Development of parental lines with high WB resistance.
- Enhancement of WB resistance using molecular technologies.
- Incorporation of WB resistance into agronomically superior and well adapted germplasm.

Initial breeding crosses were made between known WB resistant sources identified before this project and elite breeding lines from CIMMYT or Bangladesh; however, with the progress of this project, novel resistant sources were identified and utilized in the respective breeding programs.

Two types of crosses were made, i.e., 2NS x 2NS and 2NS x 2AS. The former type of cross was predominant since the frequency of 2NS is very high in recent CIMMYT international nurseries, often exceeding 90%. The advantage of 2NS x 2NS crosses was the fixation of 2NS, and thus no molecular marker work was required for diagnosing the 2NS fragment. The 2NS x 2AS type aimed to pyramid additional minor QTL with 2NS, because the ‘2AS’ parents were new sources of resistance against WB and must harbor non-2NS loci conferring WB resistance. Because 2NS was not fixed in this type of crosses, 2NS diagnosing with molecular markers was requested. Only the 2NS-positive progenies were selected since 2NS is the only stably expressed QTL identified so far and should be used as a cornerstone for WB resistance.

Additional to 2NS, two resistant donors GR119 (with WB resistance genes Rmg8 and RmgGR119) and KT020-977 (with Rmg8 only) were introduced from Japan, and crosses of the two donors with four CIMMYT recipient lines were made. A CAPS marker KM65 for Rmg8 were provided by Dr. Tosa in Kobe University, Japan, and it worked very well in the wheat pathology lab. of CIMMYT. The mapping work for RmgGR119 in Dr. Tosa's lab. is ongoing and hopefully the corresponding markers will be ready for MAS soon. These markers will be very helpful in tracking of the genes in breeding.

Both types of crosses were incorporated into the breeding pipelines in CIMMYT and Bangladesh. The recipient parents were locally adapted varieties or breeding lines with high yield potential, rust resistance and good agronomy, whereas the donor parents were WB resistant lines. The breeding strategy was similar to that for pyramiding minor genes for rusts and other quantitatively inherited traits, which have shown to be very effective in CIMMYT. Because the donor parents were not the best yielding parents, back crosses or top (3-way) crosses with higher yielding parents were made. Progenies were evaluated for agronomic traits and resistance to diseases particularly rusts in inoculated nurseries, and selected F2 and F3 plants were bulked for sowing in the next cycle, whereas from F4 onwards the selected plants were harvested and threshed individually. Similar breeding methods were adopted in Bangladesh, where targeted crosses are being made each year and selection is being made in subsequent filial evaluation. Wheat lines included national nurseries and yield trials are undergoing evaluation for wheat blast under natural and artificial inoculated condition.

The experimental stations for breeding at CIMMYT are in El Batan (for crosses), Toluca (for selection on agronomy, Septoria tritici blotch and yellow rust), and Obregon (for selection on agronomy, leaf and stem rust and yield trial). The cropping cycles in El Batan and Toluca are from May to September, and those in Obregon run from November to May. The shuttle breeding scheme in CIMMYT-Mexico enabled two cycles a year, which accelerated the breeding progress. In Bangladesh, the breeding station is in Dinajpur, and WB screening is in Jashore as mentioned before. The crop cycles in both locations run from November to March.

The accelerated breeding approaches for generation advancement was expected to be utilized, but the construction of the facilities in the Toluca station was finished in 2020 only, thus the breeding work in this project did not benefit from this facility. But in the phase II of this project, crosses and early-generation advancement will be conducted in the accelerated breeding facilities.

### 5.4 Dissemination and adaptation of improved varieties in Bangladesh

Rapid seed multiplication and dissemination are critical for the cultivation of WB resistant varieties in farmers' field to prevent the further spread of the disease. Seed multiplication was firstly conducted for the released varieties with known WB resistance or tolerance. In this regard, BARI Gom 30 (tolerant to WB and released in 2014) and BARI Gom 33 (resistant to WB and released in 2017) were the first ones that underwent seed multiplication procedure, followed by two additional varieties WMRI Gom 2 (tolerant to WB) and WMRI Gom 3 (original name "Borlaug 100", highly resistant to WB), both released in 2020.

For a newly released variety, special seed multiplication program by BWMRI was undertaken to produce reasonable quantity of breeder seed and then Truthfully Labelled Seed (TLS) for distribution. Almost all the breeder seeds are supplied to Bangladesh

Agriculture Development Corporation (BADC) to multiply as foundation and certified seed for dissemination across the country.

Fast track seed multiplication programmes were also adopted for the WB tolerant or resistant varieties for quick dissemination to the farmers. Under the present wheat seed system in Bangladesh, BWMRI is the sole institute to supply breeder seed to BADC and other private seed companies. Certified seed of a new variety takes 4-5 years to reach farmers' hand. As an alternative, BWMRI provides TLS to farmers in the same or very next year of variety release. Fast track seed multiplication programmes have been adopted to increase the seed replacement rate with newly released WB tolerant/resistant varieties. Pre-release seed multiplication could be a potential solution for rapid dissemination of new varieties seed at farmer's level, and the community-based seed production concept is very useful in this regard. According to this concept, special seed multiplication program was taken up by BWMRI to produce seed of the released varieties through progressive farmers with the involvement of Bangladesh Department of Agricultural Extension (DAE) in the process. DAE was informed about the addresses of some of the progressive farmers, so that in the coming season DAE could collect those seed for demonstration seed exchange. Clusters were selected in various districts, where TLS of the released varieties was supplied to farmers for seed multiplication, and the harvested seeds would then be used for local production. Seed multiplication trials were also used as field demonstrations to foster farmer awareness/adoption.

WB resistant or tolerant varieties (e.g., Borlaug 100 and Super152/Baj#1) and lines selected from Bangladesh (Jashore), Bolivia (Quirusillas and Okinawa), and CIMMYT trials were evaluated at multiple locations using participatory variety selection and evaluated for agronomic performance, disease response and grain yield.

For seed dissemination in the southern coastal region, this project was aligned to the project "Incorporating salt-tolerant wheat and pulses into smallholder farming systems in southern Bangladesh" funded by ACIAR and KGF, aiming at the exploration of the suitability of wheat production in salt prone areas of southern Bangladesh. BWMRI was working closely with this project. Blast resistant/tolerant varieties BARI Gom 30, BARI Gom 33 and 8 elite advance lines scored in the Jashore PPP as resistant/tolerant to WB were included in the salinity project as well, of which the varieties, BARI Gom 30, BARI Gom 33, BAW 1208 and BAW 1254 were performing well in the southern coastal region, demonstrating a promising potential to promote those WB resistant or tolerant genotypes in that region. The line BAW 1208 was released as a blast tolerant and BAW 1254 as blast resistant variety in Bangladesh in 2020. In collaboration with DAE and BARI, BWMRI had a demonstration of blast resistant or tolerant varieties in the region in 2021.

# 6. Achievements against activities and outputs/milestones

### Objective 1: To screen for WB resistance in precision phenotyping platforms (PPP)

| no. | activity | outputs/ milestones | completion date | comments |
|---|---|---|---|---|
| 1.1 | Establishment of PPPs | PPPs in Bolivia and Bangladesh fully functional | June 2018 | Three PPPs, two in Bolivia (Quirusillas and Okinawa) and one in Bangladesh (Jashore) established |
| 1.2 | Screening Asian germplasm | Materials from Bangladesh, India, Pakistan, Nepal & China screened | June 2021 | 2,681 Asian genotypes have been evaluated, some of which will be evaluated again in the phase II project to confirm their resistance against WB |
| 1.3 | Screening CIMMYT germplasm | CIMMYT nurseries including IBWSN, SAWSN, HLBSN, HPAN and IDSN evaluated (Nurseries are defined in Table 1 in Appendix) | June 2021 | 3,368 breeding lines have been evaluated, some of which will be evaluated again in the phase II project to confirm their resistance against WB |
| 1.4 | Screening synthetic wheat lines | Synthetic wheat lines evaluated in PPPs | June 2021 | 423 synthetic accessions have been evaluated |
| 1.5 | Screening miscellaneous materials | Miscellaneous breeding materials and landraces screened | June 2021 | 597 genotypes from miscellaneous nurseries have been evaluated |

### Objective 2: To decipher genetic mechanism of WB resistance

| no. | activity | outputs/ milestones | completion date | comments |
|---|---|---|---|---|
| 2.1 | QTL analysis for 10 biparental populations | Identification of non-2NS QTL and markers amenable for breeding | June 2020 | 2,910 RILs have been evaluated in multiple environments and three of them have been genotyped and analysed |
| 2.2 | GWAS for CIMMYT germplasm | Assessment of the role of 2NS in CIMMYT germplasm | June 2021 | CIMMYT breeding panels with genotypic data, 1,460 lines in total, have been analysed |
| 2.3 | GWAS for Asian germplasm | Assessment of the role of 2NS in Asian germplasm | June 2021 | Asian panels with genotypic data, 1,152 accessions in total, have been analysed and the remaining nurseries will be analysed later |
| 2.4 | GWAS for synthetic panel | Identification of a few repeatable loci | June 2020 | GWAS analysis has been done |

## *Objective 3: To breed for WB resistance*

| **no.** | **activity** | **outputs/ milestones** | **completion date** | **comments** |
|---|---|---|---|---|
| 3.1 | Breeding activities in CIMMYT | Advanced breeding lines with blast resistance | Recurrent activity | More than 100 crosses have been made and the progenies have been incorporated into the CIMMYT breeding pipeline. |
| 3.2 | Breeding activities in Bangladesh | Wheat varieties with blast resistance | Recurrent activity | Crosses involving WB resistant or tolerant varieties BARI Gom 30, WMRI Gom 3 and BARI Gom 33 have been made and incorporated into the local breeding pipeline |
| 3.3 | Pre-breeding activities | Introduction of WB resistance genes Rmg8 and RmgGR119 | Ongoing | Crosses have been made between GR119 (with Rmg8 and RmgGR119) and KT020-977 (with Rmg8 only) and four CIMMYT recipient lines. This activity will be continued in the phase II project |

## *Objective 4: Seed multiplication of WB resistant/tolerant varieties*

| **no.** | **activity** | **outputs/ milestones** | **completion date** | **comments** |
|---|---|---|---|---|
| 4.1 | Seed increase of blast resistant/tolerant varieties | Seeds of WB resistant/tolerant varieties multiplied | Recurrent activity | BWMRI has produced 20.0, 38.8, and 40.6 tons of breeder's seed of BARI Gom 30, BARI Gom 33, WMRI Gom 2 and WMRI Gom 3 in the 2018-19, 2019-20, and 2020-21 cycles, respectively.<br>BWMRI has also produced 10 tons of TLS of BARI Gom 30, BARI Gom 33, WMRI Gom 2 and WMRI Gom 3 in 2020-21 cycle. |
| 4.2 | Dissemination of seed | Seeds of WB resistant/tolerant varieties provided to farmers | Recurrent activity | The breeder's seeds of BARI Gom 30, BARI Gom 33, WMRI Gom 2 and WMRI Gom 3 mentioned above have been disseminated in the respective cropping cycles. |
| | | | | |

*PC = partner country, A = Australia*

# 7. Key results and discussion

## 7.1 Germplasm screening for WB resistance

The full list of germplasm screened in the three PPPs is provided in Table 1. Overall, 9,979 genotypes have been evaluated in the PPPs during 2017-2021, of which many have been evaluated in eight or more experiments and reliable phenotypic data have been obtained. However, evaluation for some other nurseries have just been started in 2021 and will be continued in subsequent cropping cycles. Results of several important nurseries are summarized below.

### 7.1.1. 1WBEG and 2WBEG

These were among the first WB nurseries compiled and evaluated in the PPPs, including 19 genotypes from Bangladesh, 40 from India, 28 from Nepal, and 114 from Mexico. The two nurseries were evaluated in eight field experiments in the PPPs plus a greenhouse experiment in the biosafety level-3 laboratory at USDA-ARS, Foreign Disease-Weed Science Research Unit, Fort Detrick, MD, United States.

The WB disease pressure varied greatly among experiments, with Jash18a being the lowest with a grand mean of WB index of 11.8% and Quir19a the highest of 39.3% among the field experiments (Fig. 1). The greenhouse experiment US17, however, exhibited a much higher disease pressure, showing a grand mean of WB severity of 63.6%. A bimodal distribution of the genotypes was observed in most of the experiments, except for the ones with lower disease pressure, like Jash18a and Jash18b (Fig. 1). Phenotypic correlations of WB among experiments were all significant, with r values ranging from 0.27 to 0.72. Among the experiments, experiments in Bolivia generally showed better correlation than those in Bangladesh (Table 2). WB normally showed no or low correlation with DH and PH (Table 3), this has been observed in other nurseries too (data not shown), in sharp contrast to another spike disease of wheat, Fusarium head blight, where the disease often exhibited high levels of association with DH and PH, especially the latter (Xu et al., 2020).

Genotyping with 2NS markers identified 55 2NS carriers, which exhibited significant better WB resistance in both field and greenhouse experiments, whereas the non-2NS lines exhibited much higher WB infection. Despite the general trend, there were several 2NS carriers with higher WB infection, along with some 2AS carriers showing moderate resistance (Fig. 2). As expected, the best performers were mostly 2NS lines, whereas only a few 2AS lines like SUPER152, QUAIU#1, VOROBEY, TEPOCAT89, and FRANCOLIN#1 exhibited moderate WB resistance (Table 4). Generally, the best 2NS lines performed more consistently across experiments, whereas the best 2AS lines were more subjective to environmental conditions, with wider ranges of WB index or severity (Table 4).

Results of these two nurseries have been recently published in Frontiers in Genetics (He et al., 2021).

### 7.1.2. HLBSN nurseries

Helminthosporium Leaf Blight Screening Nurseries (HLBSN), previously known as CSISA-SB nurseries, include CIMMYT breeding lines with good resistance to spot blotch and tan spot (Singh et al., 2018). Spot blotch (SB) is an important foliar disease in tropical and sub-tropical wheat areas, often happens together with WB. Both SB and WB are breeding targets for South Asian and South American breeders, thus screening HLBSN nurseries for WB will be helpful for the breeders to select genotypes resistant to both diseases.

So far, five HLBSN nurseries have been screened, and majority of the accessions exhibited very high WB resistance (Fig. 3). Although no 2NS genotyping has been done for these nurseries, it can be inferred that majority of the lines are 2NS carriers, in accordance with the high 2NS frequency in CIMMYT germplasm that will be further discussed below. This is good for breeders in the epidemic areas of both SB and WB.

### 7.1.3. IBWSN and SAWSN nurseries

The International Bread Wheat Screening Nurseries (IBWSN) and the Semi-Arid Wheat Screening Nurseries (SAWSN) represent the best performing breeding lines of CIMMYT, which generally have high yield potential, wide adaptation, good rust resistance, along with other favourable traits.

According to Juliana et al. (2019), recent international nurseries of CIMMYT have significantly increased frequencies of 2NS translocation, due to its favourable effects on rust resistance (Lr37/Yr17/Sr38) and yield potential. For the recent IBWSN and SAWSN nurseries, the frequencies of 2NS are higher than 85% based on the available GBS marker data, i.e., 50IBWSN has a 2NS frequency of 91.7%, 51IBWSN 94.1%, 52IBWSN 93.4%, 53IBWSN 93.0%, 35SAWSN 85.8%, 36SAWSN 93.7%, and 37SAWSN 85.8%. Accordingly, majority of the accessions exhibited very high WB resistance (Fig. 4), and the grand mean values of the 2NS lines were much lower than those of the non-2NS lines, e.g., 2.7% for the former and 53.3% for the latter in 51IBWSN. Nevertheless, there are a few non-2NS lines having moderate resistance (<25%) which could be considered as potential alternative sources of WB resistance (Table 5). It is noticeable that the best one SUP152/BLOUK #1*2//LONG COL AUS SELS- 10/SUP152 had a grand mean of 1.6%, which could even be regarded as highly resistant; this line has been evaluated in only four experiments, and further validation is needed. There is another possibility, i.e., a recombination event happened around the underlying gene, leading to the presence of the resistant allele of the gene (from 2NS) along with the susceptible alleles (for 2AS) of the flanking markers. This highlights the importance of fine mapping and isolation of the underlying WB resistance gene and development of its functional marker. Currently these promising non-2NS lines are being validated, and once confirmed, they'll be used in breeding and genetic studies.

Results of the 50IBWSN, 51IBWSN, 35SAWSN, and 36SAWSN have recently been published in Scientific Report (Juliana et al., 2020).

### 7.1.4. 11HPAN and 12HPAN

Zinc deficiency is a big problem in many developing countries, especially South Asian countries, leading to a series of problems like growth retardation, loss of appetite, and impaired immune function. To alleviate this problem, zinc rich wheat varieties have been promoted in South Asia, and a special nursery called 'Harvest Plus Advanced Nursery' (HPAN) is regularly released by CIMMYT, in which the accessions have high zinc content along with other desirable traits like high yielding, rust resistance etc. The 11HPAN and 12HPAN were the first HPAN nurseries evaluated for WB resistance, and just like other CIMMYT nurseries, majority of the lines exhibited high level of resistance, in accordance with the high frequencies of 2NS carriers based on GBS marker data, i.e., 96.8% in 11HPAN and 94.7% in 12HPAN. In these two nurseries, there was only one non-2NS line that exhibited good WB resistance with a grand mean of WB index of 5.0% (GID 8786706, pedigree DANPHE #1*2/3/T.DICOCCON PI94625/AE.SQUARROSA (372)//SHA4/CHIL/4/SHAKTI/5/VALI/8/TRCH/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES/6/IWA8600211//2*PBW343*2/KUKUNA/7/PBW343*2/KUKUNA*2//FRTL/PIFED). Similar to the promising IBWSN/SAWSN lines, this one will be further validated.

### 7.1.5. 50IDSN and 51IDSN

International Durum Screening Nursery (IDSN) is the durum counterpart of IBWSN, comprising the most promising durum lines in terms of yield potential, rust resistance etc. So far, two such nurseries, 50IDSN and 51IDSN, have been evaluated for WB resistance. These two nurseries exhibited very high WB infection, in sharp contrast to the above breeding nurseries of common wheat (Fig. 5), owing to the absence of 2NS. The grand mean value of 50th IDSN was 52.7% and that of 51st IDSN was 55.5%. Twelve accessions in the 50th IDSN exhibited WB values less than 30%, an arbitrary threshold used in this report for Moderately Resistant (MR), whereas only two were below this value in the 51st IDSN.

Line GID 7406259 in the 50IDSN nursery (pedigree WBD881/3/PIQUERO/AMIC//PLAYERO/PLANETA/4/TRIDENT/3*KUCUK) exhibited a very good WB resistance with a grand mean of 5.9%. After checking the pedigree, the breeder pointed out that this line must be among the rare durum lines having the 2NS translocation. Additionally, there were three lines in 50IDSN having grand mean values between 15-20% (Fig. 5) which could be used as MR sources.

### 7.1.6 Indian panels

Up to now, four Indian panels (IND100, IND353, IND350 and IND285) with 1,088 genotypes in total have been screened in the PPPs. The evaluation for IND100 and IND353 has been finished, and that for IND350 and IND285 will be continued in the coming seasons.

The histograms of the four panels indicated clear bi-modal distributions (Fig. 6), implying the important role of 2NS in these panels, which agrees with the GWAS results to be presented later. The panels IND100, IND353, and IND350 have been genotyped with markers associated with the 2NS/2AS translocation, and results indicated 2NS frequencies of 35.0% in IND100, 27.5% in IND353, and 36.9% in IND350. Just like the previously mentioned nurseries, 2NS carriers exhibited significantly higher WB resistance than 2AS carriers (Fig. 7), although there are several outliers identified in both groups. The genotyping work on IND285 is ongoing; yet the 2NS frequency might be higher than the other three panels, since around half of the lines exhibited a mean WB index lower than 20%, an empirical threshold for 2NS vs. 2AS carriers. Nevertheless, IND285 has only been evaluated in two experiments in Jashore in the 2020-21 cycle, and more experiments are needed to corroborate the phenotypic results. At the same time, this panel is being genotyped with genome-wide markers as well as the 2NS-associated STS markers.

Several non-2NS genotypes with moderate resistance to WB have been found, mostly having WB index between 20-30% (Fig. 7, Table 6). Based on the 2NS marker results, several lines appeared to have recombination in the 2NS/2AS region, and thus their 2NS status remained to be confirmed. A few of them, like UAS3006 (mean WB index 7.3%)

and UP3035 (17.0%), had very good WB resistance, and if they are proven to be 2AS carriers, then they could be used as non-2NS resistant donors.

It is worth noting that durum and dicoccum lines and two triticale lines were included in these Indian panels, accounting for 7.0%, 15.0%, 12.9%, and 10.3% in IND100, IND353, IND350 and IND280, respectively. Generally, these lines were more susceptible than bread wheat lines, but there were 20 durum lines exhibiting the 2NS alleles when tested with the 2NS-associated STS markers, and majority of them had good WB resistance, represented by AKDW2997-16 (grand mean WB index of 0), HI8819 (0.9%), DDW55 (2.0%), and PDW360 (11.7%). These durum lines could be used as WB resistant donors in durum breeding in South Asia. Interestingly, both triticale lines, TL 2969 in IND100 and TL 2969 in IND353 exhibited moderate WB resistance, with grand mean WB index of 12.5% and 25.1%, respectively. And the two STS markers WGGB156 and WGGB159 amplified 2NS-like bands in these two lines, which could be explained by 1) their durum parents have the 2NS/2AS translocation, or 2) there is a WB resistance gene in 2RS genome region homologous to 2NS. The existence of WB resistance in the triticale genepool is a good news for triticale breeding in South Asia, since triticale can also be infected by MoT, as demonstrated by Roy et al. (2021a).

### 7.1.7 Bangladeshi panels

Three panels from Bangladesh, designated BD99, BD450 and BD250, have been evaluated for WB resistance. The first two nurseries were evaluated in 12 experiments in the PPPs and BD250 was evaluated only at the Jashore PPP during the 2020-21 crop cycle. Like the Indian panels, big variation in WB resistance was found, with bi-modal distribution, too (Fig. 8). 2NS marker results indicated that 16.2% of lines in BD99 and 28.0% of lines in BD450 were 2NS carriers, and as expected, 2NS lines exhibited significantly better resistance than 2AS lines.

Apart from the many 2NS lines that exhibited very high WB resistance, there were several non-2NS exhibiting moderate resistance (Table 7). The best one was the Bangladesh line BAW-1272 with a grand mean of 11.9%, which performed relatively consistently across experiments, with the highest score of 48.4% and the lowest score of 0. Additionally, there are two more lines having mean WB index below 20%, and seven between 20-25% (Table 7). These could be used as non-2NS resistant sources in breeding programs in Bangladesh, to alleviate the strong reliance on 2NS resistance.

BD250 encompasses a set of 250 high yielding wheat genotypes with diverse sources including AYT, PYT, CVD, MLT, and also from international nurseries. Out of the 250 genotypes evaluated, around 202 genotypes were found resistant, of which 75 genotypes did not show blast infection and the rest showed <10% WB index. Around 13 genotypes displayed moderately resistant reaction (11-25% disease index) and the remaining lines exhibited susceptible to highly susceptible reaction. The resistant checks BARI Gom 33 and WMRI Gom 3 were found resistant (immune response) while the susceptible variety BARI Gom 26 displayed higher disease index (82.5%). Most of the resistant lines had 2NS translocation, however, some of the lines (BAW 1272, BAW 1286 etc.) with non-2NS background also expressed resistant reaction against the disease. These resistant 2NS and non-2NS lines will again be tested under greenhouse and field conditions for confirming their resistance and crosses will be made between lines with and without 2NS based resistance.

### 7.1.8 Pakistani panels

Being a South Asian country, Pakistan also has WB vulnerable areas in its southmost regions (Mottaleb et al., 2018). Therefore, evaluation of its wheat genotypes for WB resistance is very helpful as a preemptive strategy. Recently, two Pakistan panels, PK100 and PK128, were respectively evaluated in Jashore PPP during the 2019-20 and 2020-21 cropping cycles. Although 2NS marker data was not available, it could be inferred from the phenotypic data that around 50% of the lines were 2NS carriers, since about half of the panels exhibited a WB index less than 10% (Fig. 9). Because only two experiments have been conducted in a single location, results for this panel can only be regarded as tentative, and further validation of the promising lines is needed.

### 7.1.9 Chinese panels

There have been two Chinese panels evaluated for WB resistance.

The first one was CIMMYT-JAAS with 266 accessions, of which 132 accessions were from China, 71 from CIMMYT-Mexico, 41 from South America, 10 from North America, five each from Asia and Europe, and one each from Oceania and Africa. This panel was originally compiled for Fusarium head blight research under a collaborative project with Jiangsu Academy of Agricultural Sciences (JAAS). The panel exhibited big variation in WB resistance among accessions (Fig. 10), and average blast index of all the accessions was 53.7±12.7%, and 10 accessions including Chinese accessions “Yumai10” and “Yu02321”, all being 2NS carriers, showed moderate to high levels of blast resistance (WB index <25%), accounting for only 3.8% of the panel. Totally 23 2NS carriers were identified, exhibiting WB indices from 18.1 to 43.5%, with a grand mean index of 28.3%. None of the 2AS carriers exhibited WB indices less than 30%, and only three had values between 30-35%, i.e., Ningmai13 (32.2%), Shenhemai1 (33.2%), BUCK AUSTRAL (33.5%), and Zhoumai24 (34.3), which could be regarded as moderately resistant. Results of this panel has been published in the Crop Journal (Wu et al., 2021).

The second one was China-CAAS panel with 300 genotypes provided by Chinese Academy of Agricultural Sciences, of which most lines were Chinese winter wheat genotypes from the Yellow and Huai Wheat Production Zone, the main wheat production region in China. A normal distribution for WB resistance formed for this panel (Fig. 10), with a grand mean WB index of 43.4%. Genotyping work is being done at CAAS to investigate the association of 2NS and WB resistance in this panel, which might be similar to the CIMMYT-JAAS panel with a low 2NS frequency. Nevertheless, the best performers of this panel have ‘Milan’ (CIMMYT 2NS donor) in their pedigrees, implying the presence of 2NS in such lines.

### 7.1.10 Synthetic wheat lines

The synthetic hexaploid wheat (SHW) panel with 423 lines (Syn423) was evaluated in 12 experiments; but phenology was a great problem for this panel, because many lines were very late and tall, making WB evaluation a difficult task. WB index of this panel exhibited normal distribution (Fig. 11) with a grand mean of WB index of 47.9%, implying a typical quantitative control of this trait. There were two lines showing grand mean of WB index less than 20%, i.e. ALTAR 84/AE.SQUARROSA (333) (17.4%) and ROK/KML//AE.SQUARROSA (333) (19.7%), and there are 11 additional lines with WB means between 20-30%, being moderately resistant. No line was as resistant as the resistant checks, since 2NS is not present in the SHW germplasm pool.

### 7.1.11 Core collection of Iranian and Mexican landraces

The Iranian and Mexican landrace panel (Iran-Core with 416 lines and Mexico-Core with 181 lines) was evaluated in Quirusillas and Jashore during the 2019-20 cycle. As expected, lots of the lines were not headed or very late and thus were not evaluated. This is especially true for Jashore, where only about 150 lines were successfully evaluated. There were many lines exhibiting low WB infection, which could be caused by disease escape considering their lateness; thus, the data should be regarded as preliminary. No follow-up experiment was conducted in later cycles, and this panel could be evaluated again depending on the screening capacity of the PPPs and seed availability.

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## 7.2 Genetics of wheat blast resistance

### 7.2.1 GWAS for 1WBEG and 2WBEG

Results of GWAS for these two panels have recently been published in Frontiers in Genetics (He et al., 2021). Briefly, the 2NS/2AS translocation was the only stably expressed QTL, whereas a few more marker-trait associations (MTA) have been identified on 1BS, 6BS, and 7BL, having low phenotypic effects and being significant in only two experiments (Table 8, Fig. 12).

### 7.2.2 GWAS for IBWSN and SAWSN nurseries

Results of GWAS for 50IBWSN, 51IBWSN, 35SAWSN, and 36SAWSN have recently been published in Scientific Report (Juliana et al., 2020). The results again indicated the significant role of 2NS, whereas a few MTAs were found on 3BS, 3BL, 4AL and 7BL under limited environments, with minor effects.

### 7.2.3 GWAS for IND100&BD99, IND353, and IND350

Because IND100 is a small panel, it was combined with BD99 to perform a GWAS, and the analysis for IND353 and IND350 were conducted independently. The preliminary GWAS results for the three panels were again very similar to the above-mentioned panels, with 2NS being the only stably detected QTL with major effects. Additional MTAs with minor effects were found on 6A and 7D for IND100&BD99, 5A, 6A, and 4D for IND353, and 7B for IND350. More GWAS models will be tried to identify additional MTAs; but it is unlikely that the results will be improved significantly.

### 7.2.4 GWAS for the BD450 panel

According to the preliminary results, MTAs besides 2NS have been identified on 2B, 3A, 4A, 4D, 5A, 5B, 6A, and 7A, of which the one on 2B might be related to the known resistance gene Rmg8 (Anh et al., 2015).

### 7.2.5 GWAS for the Chinese panels

Results of the CIMMYT-JAAS panel has recently been published in the Crop Journal (Wu et al., 2021), with only the 2NS-associated markers being significant across experiments.

The China-CAAS panel is being analyzed by Dr. Yuanfeng Hao, CAAS, China.

### 7.2.6 GWAS for the Syn423 panel

Efforts have been done to optimize the GWAS analysis for this panel; but unfortunately, no success was obtained, i.e. no stable MTA could be identified, and only a few markers on 1B, 4A, 7A etc. could be identified repeatedly in limited environments, with phenotypic effects of merely 3-4%. This could be caused by three possible reasons: 1) phenotypic data were of poor quality, as reflected in the low phenotypic correlations among experiments, due to phenological heterogeneity of the SHW lines; 2) there was no MTA with major effect available in the panel, as reflected in the normal distribution of the WB index; and 3) frequencies of the favorable alleles were too low to be detected. As mentioned in the above sections, a few SHW lines did show moderate levels of WB resistance; but the underlying loci are unknown. The best case will be that one or a few loci are responsible for the resistance, which could be detected in bi-parental populations with those lines as resistant parents. But it is also possible that many loci with minor effects were contributing to the WB resistance, making future genetic studies likely to be unpromising.

### 7.2.7 QTL mapping for WBSN populations

We have made 10 bi-parental mapping populations to decipher resistance to wheat blast. After six experiments, we selected four mapping populations for additional six experiments, they are Caninde#1-Alondra, Caninde#2-Milan-S, Alondra-Milan, and Milan-Maringa. It should be noted that there were two sister lines of Milan, one having 2NS and the other not; the line used in this Caninde#2-Milan-S cross was the non-2NS line (thus named Milan-S). Up to now, the first three populations have been genotyped and here is a briefing of these populations:

**Caninde#1-Alondra**. The mapping results have recently been published in Theor. Appl. Genet. (He et al., 2020). In this population, the QTL on 2NS was found to be more closely linked to two codominant STS markers WGGB156 and WGGB159 than the frequently used dominant marker Ventriup-Ln2 (Fig. 13). This QTL was significant in all 12 experiments, exhibiting phenotypic effects from 22-51%. Several other minor QTL have been identified on chromosomes 1AS, 2BL, 3AL, 4BS, 4DL and 7BS, being significant in some of the experiments only.

**Caninde#2-Milan-S**. Since Caninde#2 is a 2NS carrier and Milan-S is of non-2NS, then 2NS was segregating in the population. In this population, the 2NS QTL was mapped mostly closer to the two WGGB markers; but in two out of the 12 experiments, the QTL peaks were closer to Ventriup-Ln2. This QTL explained 17-59% of phenotypic variation, and minor QTL were found on chromosomes 2BS, 4AL, 5AS, 5DL, 7AS, and 7AL, being significant in limited environments.

**Alondra-Milan**. The 2NS QTL was consistently closer to the two WGGB markers than to Ventriup-Ln2, explaining phenotypic effects of 26-79%. Several minor QTL have been identified on 2DL, 7AL, and 7DS, accounting for 4-9% of phenotypic variation.

### 7.2.8 The association of WB resistance with Ventriup-Ln2 and the two WGGB markers

Although the WB resistant QTL on 2NS appeared to be closer to the two WGGB markers than to the Ventriup-Ln2 marker in all the above mentioned bi-parental populations, it was the latter marker that showed better association with WB resistance in a big panel of 1,200 wheat lines (Fig. 14). This is especially true in the Indian lines, probably due to a founder parent with the susceptible alleles of the two WGGB markers being linked to 2NS. This problem could be solved with the future fine mapping work. Nevertheless, lines with the resistance alleles from both Ventriup-Ln2 and the WGGB markers had the lowest mean WB index, which is understandable since the WB QTL was mapped in between the markers and using both the flanking markers always gives better predictability than using marker from only one side.

### 7.2.9 Mapping for non-2NS WB resistance

In order to map several non-2NS resistant sources identified in this project, nine crosses were made (Table 9), where the female parents were all non-2NS carriers with moderate resistance to WB, and the male parents were non-2NS carriers with high susceptibility to WB, in the hope of identification of non-2NS QTL with major effects that can be used in breeding. The crosses were made in CIMMYT's CENEB station in the 2019-20 cycle, and the F1 seeds were sown in the El Batan station for advancement during the 2020 summer cycle. F2 plants were grown in the Agua Fria station during the 2020-21 cycle, and F3 lines have just been harvested in the El Batan station in the 2021 summer cycle.

## 7.3 Breeding for wheat blast resistance

Breeding for resistance to WB was initiated in 2017 summer season in El Batan by making 100 simple crosses using two lines identified to carry the highest level of resistance to blast in previous studies, 'SRN/AE.SQUARROSA(358)//FILIN/MILAN/3/GONDO' and 'MILAN/MUNIA' and high yielding lines that have shown good performance in Bangladesh or eastern Gangetic plains of India. Because the two donors are not the best yielding parents, we made back crosses or top (3-way) crosses with higher yielding parents during the 2017-18 crop season in Cd. Obregon. Ninety-nine F3 populations were grown during the 2019 summer season at Toluca, plants selected for agronomic traits and resistance to diseases particularly yellow rust in inoculated nurseries and harvested as bulk to obtain F4 populations. The 99 F4 populations were sown during the 2019-20 main season at Obregon, selected for agronomic traits and resistance to leaf rust in inoculated nurseries. Selected plants were harvested and threshed individually and selected for grain traits. Under normal circumstance the F5 lines would have been sown in Toluca during the 2020 summer season, however COVID-19 caused change in plans, and they were actually grown in Obregon during the 2020-21 season after phenotyping for grain to select for agronomic traits, uniformity, and leaf rust resistance. In total 2,575 small plots were grown in Obregon during the 2020-21 season, and selection was carried out for agronomic traits and rust resistance and about 750 plots retained. After harvesting and grain selection, about 300 F6 lines were advance for planting at El Batan and Toluca research station during the 2021 season for further phenotyping on agronomic traits and resistance to yellow rust. We now have 118 lines left, which will be harvested and go through genomic selection for advancement to Stage 1 yield trials.

To diversify the WB resistance available in CIMMYT germplasm via adding non-2NS resistance into the 2NS background, seven crosses between 2NS lines (resistant to WB) and non-2NS lines (moderately resistant to WB) were made in Obregon during the 2019-20 cycle (Table 10), in the hope to pyramid minor QTL with 2NS to achieve better WB resistance. The F1 seeds were sown in Obregon during the 2020-21 cycle for top/back crosses with high yielding parents (due to the COVID reason as stated above, this was not conducted in Toluca during the 2020 season). The F3 populations have recently (the

2021 season) been harvested in Toluca after screening for agronomic traits and yellow rust resistance.

The Rmg8 carrier KT020-977 was successfully crossed with two CIMMYT lines and F1 seeds have been sown in the greenhouse in El Batan for backcrosses with the recipient parents. Its crosses with two additional CIMMYT lines have been made in the greenhouse in El Batan and the F1 seeds will be harvested soon. The crosses with another resistant source GR119 have been made, too, in the greenhouse in El Batan. This was delayed due to the extremely late phenology of GR119 that made the crosses failed in 2020.

In Bangladesh, hybridization between BARI Gom 30 (blast tolerant and non-2NS) and BARI Gom 33 (blast resistant and 2NS) started in 2016 and so far, the most advanced lines are in F6 generation. Additional targeted crosses are being made each year for developing germplasm with enhanced resistance to WB involving WB resistant or tolerant varieties or lines. BWMRI is planning to evaluate the segregating generations from the blast targeted crosses in Jashore if additional land is secured. At present moment only advance lines and elite germplasms are being evaluated at Jashore for WB resistance.

Bangladesh Wheat Screening Nursery (BWSN-1) is a national nursery which includes selected wheat lines from F6 generation of national hybridization program. In 2021, 14 genotypes with wheat blast index 0-10% were selected.

Eight advance lines were selected in Preliminary Yield Trial (PYT) in 2020-21 crop cycle. Six of them belong to non-2NS and two to 2NS group. The non-2NS group includes BAW 1402 (WB index: 7.45%), BAW 1403 (2.13%), BAW 1406 (34.25%), BAW 1408 (0%) and BAW 1422 (1.06%). 2NS group include BAW 1411(11.9%) and BAW 1425 (8.8%). Five advance lines, BAW 1390 (4%), BAW 1394 (4.1%), BAW 1397 (2.3%), BAW 1399 (3.7%) and BAW 1401(2.8%), were selected in Advance Yield Trial (AYT), 2020-21 where all of them are 2NS positive.

## 7.4 Dissemination and adaptation of improved varieties in Bangladesh

Since the 2016 WB outbreak, two resistant wheat varieties have been released by BWMRI, i.e., BARI Gom 33 released in 2017 and WMRI Gom 3 in 2020. BARI Gom 30 released in 2014 was found tolerant to WB over the years. Another wheat blast tolerant variety WMRI Gom 2 was released in 2020 for commercial cultivation. All the four varieties are in the process of variety maintenance and breeders seed production. BWMRI has seed production program in its different stations and farmers field under close supervision. Tables 11 and 12 show the seed production status of BWMRI in recent years.

In 2021, four clusters were selected in Thakurgaon and Dinajpur districts for community-based seed production and TLS of BARI Gom 33 was supplied to farmers of the four clusters. BARI Gom 33 was grown in 10 bigha (330 decimal) in each cluster. It made more than 22 tons seed available to those areas.

Blast resistant or tolerant varieties BARI Gom 30, BARI Gom 33 and 8 elite advance lines scored in the Jashore PPP as resistant or tolerant to WB have been included in the Salinity Benchmark trial ran by the salinity project “Incorporating salt-tolerant wheat and pulses into smallholder farming systems in southern Bangladesh”. BARI Gom 30, BARI

Gom 33, WMRI Gom 2 and WMRI Gom 3 are performing well in the southern coastal region. It is good feedback from the salinity project to promote the WB resistant varieties in coastal region. We have good linkage with KGF to demonstrate the performance of the new blast resistant or tolerant varieties in the south. BWMRI had demonstration and seed production program of BARI Gom 30 and BARI Gom 33 in Patuakhali and Shatkhira districts under this project. As for the current project, there were 15 demonstrations in Meherpur, Rajbari and Faridpur districts with BARI Gom 33 and WMRI Gom 3. No wheat blast was reported in those demo plots. Around 25 tons of quality seed was produced in those districts.

DAE has their own seed production program under different projects in southwestern and southern districts.

# 8 Impacts

## 8.1 Scientific impacts – now and in 5 years

2NS has usually been taken as a chromosome segment that does not recombine with wheat 2AS, and thus the marker Ventriup-Ln2 has been regarded as diagnostic for the translocation. Our results exhibited the occurrence of recombination, raising the concern of the predictability of this marker. Although our haplotyping results indicated that Ventriup-Ln2 has good predictability, its combination with the two STS markers significantly increased the predictability. Based on these findings, fine mapping of the gene is justified, although lots of difficulties is expected, of which the low recombination rate is the biggest challenge. Anyway, our work has laid foundation for the fine mapping work, and better markers for 2NS might be generated in the subsequent work, which will in turn significantly contribute the WB resistance breeding.

Although no non-2NS WB resistant source that is comparable to 2NS has been identified, we did identify several moderately resistant lines, which could be utilized in future breeding activities, as well as in genetic studies to identify non-2NS QTL. Nevertheless, it is alarming that the non-2NS resistant sources were of very low frequency after screening nearly 10,000 genotypes in this project. And considering the fast evolution of the MoT pathogen, it is imperative to screening more germplasm from the primary, secondary or even tertiary gene pools of wheat to identify resistant sources that could be used in pre-breeding and breeding activities.

## 8.2 Capacity impacts – now and in 5 years

The WB PPPs in Bangladesh and Bolivia provide an opportunity for young breeders, pathologists, researchers, extension workers, farmers, as well as students to know the disease, to learn its evaluation and management strategies. In this regard, the PPPs are serving as an ideal training hub for WB related activities, like seminars, workshops, and training courses. They also provide great opportunities for trainees and visitors from different countries to communicate wheat-related subjects. A good example is the annual WB training course held in Jashore, Bangladesh. It has attracted trainees not only from South Asia, but also from China and Africa, exhibiting the attention on WB of the governments.

The pathology labs to provide MoT inoculum to the PPPs have been established and are fully functional. Their functions have gone beyond inoculum production, and several studies on fungal biology have been successfully conducted.

A mini greenhouse has been established at BWMRI Dinajpur with the help of ACIAR and CIMMYT. This greenhouse will help to screen elite materials during off-season to get resistance sources under high disease pressure. BWMRI scientists have started to grow seedling and hopefully will inoculate those materials in greenhouse soon. BWMRI scientists are also going to develop differential lines for wheat blast inside this greenhouse.

With support from several funding agencies including ACIAR-Australia, CRP WHEAT, SRC-Sweden, USAID-USA, ICAR-India, and KGF-Bangladesh, several trainings were organized, both formal and informal.

Formal training activities:

In July 2017, a two-week hands-on training on wheat blast targeting mainly disease screening but including pathogen characterization and blast identification, host resistance and disease management strategies, and glasshouse and in-field screening and data recording was organized by CIMMYT. Ten trainees from Bangladesh, India, Nepal and Mexico participated in the training, which started at USDA-ARS, Fort Detrick, USA on molecular marker diagnosis on MoT, greenhouse screening for blast resistance and global concern on wheat blast. The trainees also had a tour to the Biosafety Level-3 Containment greenhouses with observation of plant inoculation methods and disease evaluation. In Bolivia, the trainees had an opportunity to do field disease evaluation in the CAICO (Okinawa), ANAPO and CIAT experiment stations. They also visited the blast-screening nursery in Quirusillas.

An international training course on WB disease screening and surveillance was regularly organized at the Regional Agricultural Research Station, Jashore, Bangladesh in late February or early March yearly from 2017-2020 (suspended in 2021 due to COVID-19 pandemic). The objective of the training was to learn the basic techniques of pathogen identification and its culturing, WB screening including field inoculation and disease scoring, field surveillance to the blast-affected wheat fields in Jashore and Meherpur regions and share the experience regarding combating the disease and its progress among the participants from home and abroad. Totally 100 wheat scientists from India, Zambia, Afghanistan, China and Nepal as well as from BWMRI, BSMRAU, BAU, DAE and CIMMYT in Bangladesh participated in the training. Detailed and practical hands-on training was provided at the PPP for WB where germplasm from different countries of the world and CIMMYT-Mexico are being tested under artificial inoculated condition. Crucial aspects of WB screening, pathogen biology, WB management, monitoring and surveillance and wheat blast forecasting model was presented and discussed. In 2020, participants also visited BSMRAU in Gazipur, where Dr. Tofazzal Islam briefed about mutation breeding and the use of nano technology and probiotic bacteria in controlling wheat blast.

Dr. M.R. Kabir participated in a twelve-week (16 October 2018 to 15 January 2019) USDA-Borlaug fellowship program for research on WB at Purdue University, USA and ANAPO, Santa Cruz, Bolivia.

Informal training activities:

Twelve batches of training on "Wheat blast disease and its management" were organized in 2019 in different regions of Bangladesh including Jashore, Meherpur, Faridpur, Kustia, Bhula and Gazipur. Researchers, Extension personnel and farmers from respective regions participated in the training program.

BWMRI scientists have given several informal training activities at PPP Jashore and farmer’s field. Sometimes students from university and colleges came to visit the PPP to gather and upgrade their knowledge on wheat blast. Scientists working at Jashore station have given informal lecture to them about wheat blast disease. Also, those scientists visited farmers’ fields and have given practical idea about the disease management technology which is much helpful for the farmers to manage it successfully.

One researcher from Bolivia, Mr. Jose Asister visited CIMMYT-Mexico from 7th October to 10th November 2018 for training in wheat pathology on fungal identification, isolation and culturing and greenhouse experimentation techniques.

In 2019, three researchers from BWMRI, Dr. Muhammad Rezaul Kabir, Mr. Krishna Kanta Roy and Mr. Babul Anwar, visited precision phenotyping platform for WB in Okinawa, Bolivia from 8th July to 10th August and CIMMYT-Mexico from 10th August to 12th September with the objective of getting hands-on training on wheat breeding and pathology targeted for wheat blast.

Two researchers from India, Dr. Naresh Kumar (IARI) and Dr. Vikas Gupta (IIBWR) visited the precision phenotyping platform for WB in Quirusillas, Bolivia from 2nd to 12th March 2019 to get hands on training on screening for wheat blast.

Ms. Tonusree Roy, a research associate in from BWMRI, visited Australia and attended the Australasian Plant Pathology Society Conference (Melbourne, 25-28 November 2019) with support of ACIAR.

Ms Ingrid Abastoflor and Mr. Jhonny Villagomez, researchers from INIAF Bolivia, visited CIMMYT-Mexico from 1st to 31st October 2019 for training in wheat quality and pathology targeted to fungal identification, isolation and culturing and greenhouse experimentation techniques.

Two researchers from India, Dr. Rahul Phuke (IARI) and Dr. Santosh Bishnoi (IIBWR) and one from China, Dr. Kaijie Xu (CAAS), visited the PPP in Okinawa, Bolivia in the first fortnight of July 2019 to get hands on training on screening for wheat blast.

Three researchers from BWMRI-Bangladesh, Dr. Akbar Hossain, Mr. Krishna Kanta Roy and Md. Babul Anwar, and Dr. Felix Marza from INIAF-Bolivia attended and presented at the 1st International Wheat Congress, 22-26 July 2019, Saskatoon, Canada.

Eleven trainings on "Wheat blast disease and its management" were organized in different regions of the country including Jashore, Meherpur, Faridpur, Kushtia, Bhola and Dinajpur. Researchers, extension personnel and farmers from respective regions participated in the training/workshop program.

## 8.3 Community impacts – now and in 5 years

### 8.3.1 Economic impacts

WB has created a negative economic impact on wheat production of Bangladesh. WB reported districts occupy 55-60% wheat area in Bangladesh. After 2016 outbreak, wheat area and production both declined in those districts which had an impact on national wheat production in last three years. Releasing of resistant variety, awareness workshop, training, demonstration, field day, coordination meeting with stakeholders, distribution of factsheets etc. helped increase the production in those districts that contributed to the national production. Wheat area and production rebounded markedly in the last years. Wheat productivity has also increased due to adoption of resistant cultivars and improved technologies. Government of Bangladesh intervention and encouragement resulted in wheat farmers getting good price (approx. 27.5 Tk/kg) which is much higher than previous (approx. 17.5 Tk/kg). Due to high input cost and low market price, boro rice (competitor of wheat) became non-profitable. Government of Bangladesh is also encouraging farmers to replace boro rice by wheat in high and medium high land and areas where frequent irrigation is needed. Government is also trying to push wheat in non-traditional areas specially Sylhet region (Eastern region) and Southern part of Bangladesh. The availability of high-performance WB resistant wheat varieties will be critical for these initiatives to succeed.

### 8.3.2 Social impacts

Upon the outbreak of WB in Bangladesh, numerous farmers suffered from significant yield reduction, and the government discouraged the cultivation of wheat in the subsequent years simply due to the fear of WB epidemic. With the development of resistant/tolerant varieties, the government and farmers re-gain the confidence of growing wheat in WB epidemic regions, which is beneficial in increasing income of farmers because wheat is an important crop for winter season and provides good economic income in Bangladesh (specially with increased price). The utilization of WB resistance varieties also means the reduced application of fungicide, which is beneficial in reducing the income gap between poor and rich farmers.

Another social impact of this project is to promote gender equality. Through the training and extension component of this project, more women researchers, students, and farmers had chance to participate the training courses, enabling women to participate in wheat research and production more actively. Through the Bangladesh research partners, the project is encouraging young women scientists to participate in the training courses.

### 8.3.3 Environmental impacts

Like the management of other wheat diseases, fungicide has been heavily relied on to control WB, especially when the resistant varieties are not available. The application of fungicide could cause social problems and environment problems as most of them can be hazardous to environment, human and animals if not used properly. As seen in South America, with the frequent application of fungicides, their efficiencies reduce over time due to development of fungicide resistance in the WB pathogen. The WB resistant wheat varieties developed in this project will lead to the reduced reliance on fungicide and therefore significantly contribute to the protection of environment.

## 8.4 Communication and dissemination activities

### 8.4.1 Demonstration of WB resistant varieties

Field demonstration of wheat blast resistant and tolerant varieties were conducted in WB prone areas. BARI Gom 33 was used in 380 demonstration plots, BARI Gom 30 in 45 plots, and WMRI Gom 3 in 10 plots in 21 WB vulnerable districts. The size of each demo plot was 40 $m^2$.

Number of field days were conducted in strategic demonstration plots. Both farmers and DAE personnel expressed their satisfaction with the performance of BARI Gom 33 and WMRI Gom 3. Farmers like these varieties because of their resistance to WB, long and attractive spike, strong stem and good productivity and demanded its seed for next year. BARI Gom 30 is also getting popular among the farmers with increased demands for it.

### 8.4.2 Blogs, infographics and media reports

**News in blog, infographics and media reports:**

1. International wheat blast training on germplasm screening and field surveillance of wheat blast at Jashore 1-10 March 2020. (Web link)

2. New infographic highlights an early warning system for wheat blast in Bangladesh. (Web link)

3. Mid-Term Review of the ACIAR funded wheat blast project, Dinajpur, 4-5 September 2019. (Web link)

4. News video in Bangla on trainee's field visit to identify wheat blast resistant germplasm performance in farmers field. Meherpur 7 March 2020. (Web link)

5. Call for action on wheat blast threat in South Asia. (Web link)

6. Assessing the effectiveness of a "wheat holiday" for preventing blast. (Web link)

7. Tracking the Weather, A New System Can Protect Brazilian Farmers from Wheat Blast. (Web link)

8. The case for rushing farmer access to BARI Gom 33, a blast resistant wheat variety released in Bangladesh. (Web link)

**Popular article in newspaper:**

1. News report in Bangla on observation of international women's day 2021 at PPP, RS, BWMRI, Jashore. (Web link)

2. News on demonstration of wheat blast resistant varieties at farmers field and field day, 25 March 2021. Field day was held with the help of DAE at Gangni, Meherpur. BARI Gom 33 had a good performance showing no blast disease. Farmers are committed to keep and exchange wheat seed for next year. (Web link)

3. Farmers training on wheat cultivation and wheat blast management on 26 November 2020 at Meherpur. Farmers were given necessary inputs for conducting the demonstration. Seed of BARI Gom 33 and WMRI Gom 3 were supplied. (Web link)

4. News on release of blast resistant wheat variety WMRI Gom 3 in a national daily. (Web link)

5. News on field day on BARI Gom 33 at Magura. (Web link)

6. The news was published in 14 February 2021 in a national daily. Wheat production becoming popular due to release of new wheat varieties. Newly released WMRI Gom 2 and WMRI Gom 3 is giving hope as they are blast resistant. (Web link)

7. News on wheat expansion in Naogaon. Wheat area became double in Raninagar. Last year wheat was cultivated in 550 ha which became 710 ha this year. Wheat area increased and farmers are expecting good harvest as they used modern wheat varieties and disease infestation is very low. (Web link)

8. A field day was held on 31 March at Ranisankail upazil of Thakurgaon District. DAE and BWMRI arranged the field day to demonstrate the performance of the variety. (Web link)

9. News report in Bangla on trainee's field visit to identify wheat blast resistant germplasm performance in farmer field. Meherpur 7 March 2020. (Web link)

10. Published in 25 January 2020. Wheat was cultivated in 11,400 ha in Meherpur which is 3,600 ha higher than the target (Source: DAE). BARI Gom 24, BARI Gom 26, BARI Gom 30, BARI Gom 33 were cultivated in Meherpur. Wheat blast was reported in Meherpur in late January 2020. Scientists from BWMRI along with DAE representative visited wheat blast infected fields. Wheat blast was observed in BARI Gom 24 and BARI

Gom 26 fields. At the same time no blast was found in BARI Gom 33. Farmers were given necessary suggestions to control the effect of blast. (Web link)

**TV news clip:**

1. Wheat production is increasing countrywide with the releasing of modern wheat varieties specially WB resistant varieties. Even we have some limitations in seed production. We cannot supply enough seed to the farmers and we are trying our best, said DG, BWMRI. (Web link)

2. Meherpur farmers are expecting bumper wheat production this year. Last 4 years wheat production was affected by blast disease. After arrival of BARI Gom 33 wheat production is bouncing back in this region. DAE is keeping eye on WB management and expansion of resistant varieties in this region. (Web link)

3. TV News24 in Bangla broadcasted on 20 February 2020. Wheat cultivation was almost stopped in last few years due to the outbreak of wheat blast in Jashore region. After releasing of resistant variety BARI Gom 33, farmers bounced back to cultivate wheat. As part of government incentive, seeds and fertilizers have been distributed to 1,200 farmers. Farmers are shifting from boro rice to wheat because of high input cost and low price of rice. Wheat cultivation is more profitable than rice. Farmers growing varieties other than BARI Gom 33 were being monitored by DAE to spray their crop in time to protect against the fungus. Wheat was cultivated in 35,736 ha in six districts of this region (Jashore, Magura Jhenaidah, Kustia, Chuadanga and Meherpur) against a target of 28,849 ha. (Web link)

4. TV News24 in Bangla broadcasted on 2 March 2020. PPP activities during wheat blast training course were broadcasted in this news. BWMRI with the help of CIMMYT and ACIAR has been working to identify the resistant wheat germplasm for last three years. They have been successfully identified and able to release a resistant variety BARI Gom 33. BARI Gom 26 will be replaced with resistant variety said Ad. Secretary. Trainees from India, Nepal, Afghanistan, and Zambia participated the in training course. (Web link)

5. This is a little elaborated news of training activities at PPP in Jashore broadcasted in Channel I on 2 March 2020. (Web link)

6. TV news telecasted on Channel 24 on 27 June 2020. BWMRI has taken initiative to supply seed of blast resistant wheat variety BARI Gom 33. Breeder seed has been supplied to BADC last year and BADC is multiplying it in its farm. BWMRI is planning to supply about 27 tons breeder seed of BARI Gom 33 to BADC. Farmers prefer this variety as it is blast free and wheat cultivation is getting popularity as it is profitable now a days. (Web link)

7. This link contains the news of MTR held at BRAC Centre, Dinajpur during 5-6 September 2019. The news was broadcasted on a national news channel (Channel I). MTR was organized by BWMRI to discuss the status of wheat blast research and future plans. Representative from CIMMYT, ACIAR, INIAF, MoA, DAE, BADC, SCA, Seed companies, NGOs participated the meeting. Results and plans were discussed in the meeting. (Web link)

8. Bangladesh national TV channel BTV has broadcasted detailed story on the research activities at PPP for WB on 3 April 2019. (Web link)

9. Different electronic media broadcasted the news of 2019 training program at the following links. (Web links 1, 2, and 3)

### 8.4.3 Journal Articles and Book Chapters

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### 8.4.4 Oral/Poster Presentations:

Mustarin, K.E., et al. 2021. Present status of leaf rust of wheat and its chemical control: Bangladesh perspective. Poster presentation. To be presented in 2021 BGRI Virtual Technical Workshop, October 6-8, Cornell University, Ithaca, New York, USA.

Singh, P.K. and J.M. Fernandes. 2019. Wheat Blast. Oral Presentation. G20 MACS-International Workshop on Facilitating International Research Collaboration on Transboundary Plant Pests. November 27-29, Tsukuba, Japan.

Singh, P.K., et al. 2019. Mitigating the threat of wheat blast-opportunities and challenges. Poster Presentation. 1st International Wheat Congress. July 22-26, Saskatoon, Canada.

Juliana, P., et al. 2019. Extending the frontiers of genomic selection, trait genetic architecture and genomic fingerprinting in CIMMYT's bread wheat breeding program. Poster Presentation. 1st International Wheat Congress. July 22-26, Saskatoon, Canada.

Roy, K.K., et al. 2019. Integrated phytopathological intervention of wheat blast-a new disease in South Asia. Poster Presentation. 1st International Wheat Congress. July 22-26, Saskatoon, Canada.

Marza, F., et al. 2019. Resistance to wheat blast among cultivars under greenhouse conditions. Poster Presentation. 1st International Wheat Congress. July 22-26, Saskatoon, Canada.

Roy, K.K., et al. 2019. Wheat blast: prevelance, epidemiology, host status and screening of elite germpalsm for resistance. Poster Presentation. 1st International Wheat Congress. July 22-26, Saskatoon, Canada.

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Barma, N.C.D., et al. 2019. Breeding for wheat blast resistance in Bangladesh: Research Progress and Future Stategies. Oral presentation. The 11th Biennial Conference of Plant Breeding and Genetics Society of Bangladesh. December 28-29, BARC, Farmgate, Dhaka, Bangladesh.

Singh, P.K., et al. 2018. Wheat Blast: current status and progress in phenotyping. 57th All India Wheat and Barley Researchers Workers Meet. August 24-26, BAU Ranchi, India.

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Cruppe, G., et al. 2018. First report of non-2NS resistance to wheat head blast. Poster Presenation. International Congress of Plant Pathology: Plant Health in A Global Economy. July 29-August 3, Boston, USA.

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Farhad, M., et al. 2018. Wheat disease surveillance and monitoring in Bangladesh. Poster Presentation. BGRI Technical Workshop, April 14-17, Marrakech, Morocco.

### 8.4.5 Field days

Bolivian National Day of Wheat, 27 July 2018 at Okinawa, SCZ, Bolivia

Research Activities Demonstration Day, 30 March 2019 at Quirusillas, SCZ, Bolivia

Advances in Blast Research Activities of INIAF, 13 June 2019 at Okinawa, SCZ, Bolivia

BARI Gom 33 field demonstration, March 14 2019 at Dighippara village, Meherpur. Bangladesh.

Field demonstration of newly released wheat blast resistant and Zn enriched wheat variety BARI Gom 33, 4 April 2019 at Jhenaidah, Bangladesh.

“Mati o Manush” a popular Bangladesh national television program for the farmers filmed and broadcasted the field day activities.

### 8.4.6 Workshops

Two national awareness workshops entitled "Present status of wheat production and its challenges’ and “Present status of wheat and maize production, challenges and prospects” were held in BWMRI, Dinajpur, Bangladesh on 01 September 2020 and 07 November 2020, respectively. Participants included representatives from Ministry of Agriculture, BARI, BWMRI, DAE, BADC, CIMMYT-Bangladesh, seed companies, farmers, seed & pesticide dealer, and media etc. Another regional workshop on “Expansion of wheat in non-traditional areas under stress environment” in Rajshahi on 19 February 2021.

Four regional awareness workshops on "Wheat blast management and boosting yield" were held in 2019 in Jashore, Rajshahi, Faridpur and Gazipur. Participants included representatives from different organizations including Ministry of Agriculture, BARI, BWMRI, DAE, BADC, CIMMYT-Bangladesh, seed companies, farmers, seed & pesticide dealer, and media etc.

Eight regional workshops were held at different agricultural region on awareness building on wheat blast disease and its management strategies in 2018.

Regional Workshop and Launching of ACIAR Funded Project on Wheat Blast was held on July 13-14, 2017 at Dhaka, Bangladesh.

### 8.4.7 Co-ordination meeting

Three co-ordination meetings were organized on "Prevalence and variability of *Magnaporthe oryzae* pathotype *triticum* causing wheat blast and screening of wheat germplasm against the disease". The first meeting was held at BARI, Gazipur on 04/11/2018, the second was organized by BWMRI, Dinajpur on 02/11/2019, and the third was held at Bangladesh Agricultural Research Council (BARC), Dhaka on 09/05/2019. Researchers and policy makers from BSMRAU, BAU, BARC, KGF and BWMRI participated in the meeting.

The Mid-Term Review meeting of the current project was organized by BWMRI and held in Dinajpur from Sept. 5-7, 2019. Participants were from ACIAR, BARI, BWMRI, DAE, BADC, KGF, INIAF, CIMMYT, seed companies, media, etc.

### 8.4.8 Leaflet distribution

Leaflet on production technology of BARI Gom 33 and WMRI Gom 3 was distributed to the wheat farmers. The leaflet was also published and distributed from Regional Station, BWMRI, Jashore on “Management of wheat blast using modern technologies in southern districts of Bangladesh”. A fact sheet was developed soon after wheat blast reporting in 2016 and it was revised several times and distributed throughout the country. Agriculture Information Service (AIS), DAE and BADC also published similar fact sheet in subsequent years. A booklet on “Wheat diseases and their management” was published by Plant Pathology Division, BWMRI and distributed throughout the country.

### 8.4.9 Farmers training on WB management

More than 2500 farmers including 250 frontline extension agent of DAE have been provided training on wheat blast management over last four years.

# 9. Conclusions and recommendations

## 9.1 Conclusions

Wheat blast has been spreading in Bangladesh in recent years despite the unfavourable weather conditions, which poses a great threat to the wheat production in the country and can lead to great losses in future years with conducive weather conditions. Fortunately, the risk from this disease has been well acknowledged in Bangladesh as well as the global wheat community, and synergic efforts are being made to reduce the potential negative impacts. In this project, nearly 10,000 wheat accessions have been screened in the three precision phenotyping platforms. Most resistant genotypes carry the 2NS/2AS translocation segment, demonstrating a very narrow genetic diversity in terms of blast resistance. And those non-2NS genotypes with resistance or moderately resistance did not perform stably across experiments, implying that their resistance must be highly influenced by environmental conditions, which is unfavourable for breeding of widely adapted genotypes with blast resistance. Genetic studies led to similar conclusion, i.e., only the 2NS/2AS translocation locus exhibited stable blast resistance across experiments, whereas other loci detected were significant only in limited environments, and none of them showed major effects. Due to a lack of non-2NS WB resistant source, resistance breeding at this stage had to rely on the 2NS/2AS translocation only, although several crosses between 2NS and non-2NS genotypes aiming to pyramid WB resistant loci have been made. The multiplication and dissemination of WB resistant or tolerant varieties in Bangladesh is going well and the areas sown to such varieties have been steadily increasing, protecting the farmers from future WB epidemics.

## 9.2 Recommendations

Despite the achievements, we must realize that the MoT pathogen keeps evolving, and the frequency of 2NS-virulent MoT isolates has been increasing rapidly in South America, and this will happen in South Asia too. Therefore, it is paramount to identify and utilize novel non-2NS resistant sources in breeding. Wheat accessions from other regions, as well as wild relatives of wheat, could be screened for such resistant sources. At the same time, the non-2NS sources identified in this project need to be actively utilized in breeding programs to alleviate the high directional selection pressure that 2NS is applying upon Bangladesh MoT isolates. Besides, possible recombination events have been found in the 2NS/2AS translocation region, which led to contradictory diagnostic results when different 2NS-associated markers were used. Precision mapping work on this chromosomal region will help to further narrow down the underlying gene and identify better markers, to facilitate the breeding and gene cloning work.

The WB research and breeding work in South America has clearly shown that growing resistant varieties is insufficient to effectively manage this disease, and other management tools, especially fungicide application and sowing date adjustment, need to be incorporated in the integrated blast management system to obtain a satisfactory control of the disease.

Apart from the abovementioned technical aspects, socioeconomical studies like farmers' preference on WB resistant variety and the impact of gender on wheat variety selection and cropping practices are also critical to counteract the obstacles during the scaling of

WB resistant varieties. All these research subjects will be addressed in the phase II project.

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## List of publications produced by project

Already listed in 8.4.3

# Appendixes

## Appendix 1: Tables and Figures

**Table 1**. Germplasm evaluated for wheat blast during 2017-2021

| Acronym | Full Name | No. of Lines | No. of Exp. |
|---|---|---|---|
| 1WBEG | 1st Wheat Blast Elite Germplasm | 100 | 8 |
| 2WBEG | 2nd Wheat Blast Elite Germplasm | 101 | 8 |
| 8HLBSN | 8th Helminthosporium Leaf Blight Screening Nursery | 50 | 8 |
| 9HLBSN | 9th Helminthosporium Leaf Blight Screening Nursery | 50 | 6 |
| 10HLBSN | 10th Helminthosporium Leaf Blight Screening Nursery | 50 | 6 |
| 11HLBSN | 11th Helminthosporium Leaf Blight Screening Nursery | 50 | 6 |
| 12HLBSN | 12th Helminthosporium Leaf Blight Screening Nursery | 50 | 4 |
| 35SAWSN | 35th Semi-Arid Wheat Screening Nursery | 277 | 12 |
| 36SAWSN | 36th Semi-Arid Wheat Screening Nursery | 300 | 12 |
| 37SAWSN | 37th Semi-Arid Wheat Screening Nursery | 283 | 8 |
| 50IBWSN | 50th International Bread Wheat Screening Nursery | 283 | 12 |
| 51IBWSN | 51st International Bread Wheat Screening Nursery | 300 | 12 |
| 52IBWSN | 52nd International Bread Wheat Screening Nursery | 284 | 10 |
| 53IBWSN | 53rd International Bread Wheat Screening Nursery | 284 | 4 |
| 11HPAN | 11th Harvest Plus Advanced Nursery | 280 | 2 |
| 12HPAN | 12th Harvest Plus Advanced Nursery | 290 | 4 |
| IND100 | 2017 Indian panel with 100 entries | 100 | 14 |
| IND353 | 2018 Indian panel with 353 entries | 353 | 12 |
| IND350 | 2019 Indian panel with 350 entries | 350 | 6 |
| IND285 | 2020 Indian panel with 285 entries | 285 | 2 |
| BD99 | Bangladesh panel with 99 entries | 99 | 12 |
| BD450 | Bangladesh panel with 450 entries | 450 | 12 |
| BD250 | Bangladesh panel with 250 entries | 250 | 2 |
| PK100 | Pakistan panel 100 lines | 100 | 2 |
| PK128 | Pakistan panel 128 lines | 128 | 2 |
| CIMMYT-JAAS | CIMMYT and Chinese panel for GWAS | 266 | 12 |
| China-CAAS | Chinese winter materials | 300 | 14 |
| Syn423 | Synthetic panel | 423 | 12 |
| 1WBSN* | 1st Wheat Blast Screening Nursery | 2910 | 12 |
| 51IDSN | 51st International Durum Screening Nursery | 159 | 10 |

| | | | |
|---|---|---|---|
| 50IDSN | 50th International Durum Screening Nursery | 177 | 10 |
| Iran-Core | Core collection of Iranian landraces | 416 | 4 |
| Mexico-Core | Core collection of Mexican landraces | 181 | 4 |
| | **Total number of entries** | 9,979 | |

Note: the entire population of 1WBSN was evaluated in six experiments, and then only a subset was evaluated in six additional experiments.

**Table 2** Pearson correlation coefficients of wheat blast index for 1WBEG and 2WBEG among the 9 environments.

| | **US17** | **Quir18a** | **Quir18b** | **Jash18a** | **Jash18b** | **Oki18a** | **Oki18b** | **Quir19a** |
|---|---|---|---|---|---|---|---|---|
| **Quir18a** | 0.58 | | | | | | | |
| **Quir18b** | 0.50 | 0.58 | | | | | | |
| **Jash18a** | 0.35 | 0.29 | 0.48 | | | | | |
| **Jash18b** | 0.52 | 0.43 | 0.52 | 0.55 | | | | |
| **Oki18a** | 0.52 | 0.56 | 0.57 | 0.41 | 0.50 | | | |
| **Oki18b** | 0.63 | 0.63 | 0.50 | 0.30 | 0.45 | 0.56 | | |
| **Quir19a** | 0.59 | 0.63 | 0.68 | 0.35 | 0.49 | 0.62 | 0.68 | |
| **Quir19b** | 0.55 | 0.71 | 0.58 | 0.27 | 0.52 | 0.63 | 0.66 | 0.72 |

Note: all correlations were significant at *P*<0.0001. 'Quir' stands for Quirusillas, 'Jash' for Jashore, and 'Oki' for Okinawa, '18' and '19' for the 2017-18 or 2018 cycle and 2018-19 cycle, respectively, and 'a' and 'b' for the first and second sowing, respectively. 'US17' stands for the 2017 greenhouse evaluation in the United States. Cell shades change from green to red with the increase of correlation coefficients.

**Table 3** Phenotypic correlation of field wheat blast index with days to heading (DH) and plant height (PH) for 1WBEG and 2WBEG in individual experiments.

| | **Quir18a** | **Quir18b** | **Jash18a** | **Jash18b** | **Oki18a** | **Oki18b** | **Quir19a** | **Quir19b** |
|---|---|---|---|---|---|---|---|---|
| **DH** | -0.06 | -0.13 | 0.07 | 0.17* | -0.06 | 0.06 | -0.01 | -0.04 |
| **PH** | -0.13 | -0.11 | 0.13 | 0.01 | -0.07 | -0.04 | -0.24** | -0.18** |

$^*p < 0.05$; $^{**} p < 0.01$

**Table 4**. Top performing lines from 1WBEG and 2WBEG nurseries and their blast reaction in different experiments.

| **Cross** | **US.GH** | **Quir.2018** | **Jash.2018** | **Oki.2018** | **Quir.2019** | **2NS** |
|---|---|---|---|---|---|---|
| MILAN/MUNIA | 0.0 | 11.6 | 6.5 | 0.0 | 0.0 | YES |
| SUP152/3/INQALAB 91*2/TUKURU//WHEAR | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | YES |
| WAXWING/KIRITATI//KACHU | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | YES |
| SUP152/3/TRCH/SRTU//KACHU | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | YES |
| KACHU #1 | 3.3 | 0.0 | 7.8 | 0.0 | 0.0 | YES |
| HUIRIVIS | 5.6 | 0.0 | 10.0 | 0.0 | 0.0 | YES |
| BORLAUG100 F2014 | 8.3 | 0.0 | 5.0 | 0.0 | 0.0 | YES |
| MILAN/BAV92//PASTOR | 8.6 | 0.0 | 8.9 | 0.0 | 4.0 | YES |
| BAW-1260 | 11.1 | 0.0 | 0.0 | 3.8 | 0.0 | YES |
| MUTUS #1 | 13.8 | 36.0 | 0.0 | 0.0 | 0.0 | YES |
| SUPER 152 | 25.4 | 52.3 | 10.9 | 39.8 | 43.6 | No |
| QUAIU #1 | 50.5 | 61.8 | 5.5 | 25.5 | 34.3 | No |
| VOROBEY | 32.5 | 35.4 | 2.1 | 33.2 | 23.7 | No |
| TEPOCA T 89 | 25.0 | 58.8 | 0.0 | 34.5 | 12.7 | No |
| FRANCOLIN #1 | 33.6 | 47.1 | 0.0 | 36.6 | 16.5 | No |
| Resistant check (Urubo or BARI Gom 33) | 1.0 | 8.2 | 3.1 | 0.0 | 5.5 | |
| Susceptible check (Atlax or BARI Gom 26) | 100.0 | 71.8 | 31.6 | 59.8 | 74.1 | |

* Average values of two sowings presented, except for US GH (greenhouse tests US) where only one experiment was done.

**Table 5.** Top performing non-2NS lines from the recent IBWSN and SAWSN nurseries and their blast reaction across experiments.

| GID | Cross | Panel | Mean Blast* |
|---|---|---|---|
| 8241055 | SUP152/BLOUK #1*2//LONG COL AUS SELS- 10/SUP152 | 53IBWSN | 1.6 |
| 8235657 | KRONSTAD F2004/KENYA SUNBIRD//WHEAR/KRONSTAD F2004/3/WBLL1*2/BRAMBLING*2//BAVIS | 53IBWSN | 5.0 |
| 8051062 | KACHU/KINDE*2//KACHU/KIRITATI | 37SAWSN | 9.0 |
| 7402225 | NELOKI*2/4/SOKOLL//PBW343*2/KUKUNA/3/ATTILA/PASTOR | 50 IBWSN | 15.0 |
| 8044901 | SUP152/BLOUK #1//BECARD/FRNCLN | 52IBWSN | 15.1 |
| 7462124 | FRET2/TUKURU//FRET2/3/MUNAL#1/4/WBLL1*2/BRAMBLING/3/KIRITATI//PBW65/2*SERI.1B | 36 SAWSN | 15.9 |
| 8051596 | KENYA SUNBIRD/KACHU//KIDEA | 52IBWSN | 21.9 |
| 8052770 | BABAX/LR42//BABAX*2/3/SHAMA/4/TACUPETO F2001*2/BRAMBLING/5/BORL14 | 52IBWSN | 22.3 |
| 8048437 | CHRZ//BOW/CROW/3/WBLL1/4/CROC_1/AE.SQUARROSA (213)//PGO*2/5/KUTZ | 52IBWSN | 22.8 |
| 6174860 | FRANCOLIN #1//WBLL1*2/BRAMBLING | 35 SAWSN | 23.7 |
|  | Resistant check (Urubo or BARI Gom 33) |  | 4.5 |
|  | Susceptible check (Atlax or BARI Gom 26) |  | 77.0 |

* Average values of WB index (%) across experiments are presented.

**Table 6.** Top performing non-2NS lines from Indian nurseries and their blast reaction across experiments.

| Panel | Name | No. of Exp. | Mean WB index (%) |
|---|---|---|---|
| IND353 | MACS6736 | 12 | 17.8 |
| IND100 | DBW 39 | 14 | 18.4 |
| IND350 | WH1264 | 6 | 19.3 |
| IND353 | NIAW3583 | 12 | 20.2 |
| IND353 | DBW297 | 12 | 21.1 |
| IND100 | HD 2204 | 14 | 21.5 |
| IND350 | VL3023 | 6 | 22.1 |
| IND353 | Raj4539 | 12 | 22.6 |
| IND353 | DBW286 | 12 | 22.7 |
| IND353 | DBW 273 | 12 | 22.8 |
| IND353 | HW 1904 | 12 | 23.5 |
| IND353 | Raj4538 | 12 | 23.6 |
| IND353 | KRL423 | 12 | 23.7 |
| IND353 | WR544 | 12 | 24.0 |
| Susceptible check | | 14 | 70.2 |
| Resistant check | | 14 | 3.2 |

**Table 7.** Top performing non-2NS lines from BD99 and BD450 nurseries and their blast reaction across experiments.

| GID | Cross | Panel | Mean Blast* |
|---|---|---|---|
| 8750907 | BAW-1272 | BD99 | 11.9 |
| 8915533 | PRODIP/SW 89.5422//BAW 1051(AYT-7) | BD450 | 17.9 |
| 82138 | SW89-5124*2/FASAN | BD450 | 19.6 |
| 5596972 | SKAUZ*2/FCT´S´//VORB | BD450 | 20.6 |
| 5106867 | UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ (CV-62) | BD450 | 21.0 |
| 412388 | CIANO 79 | BD450 | 21.6 |
| 8915565 | OASIS/3*ANGRA//708E (CV-84) | BD450 | 22.3 |
| 8915720 | SOURAV/6/CS/TH.SC//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI | BD450 | 22.8 |
| 6775597 | CHINA-01 | BD450 | 23.2 |
| 8915950 | CMH74A.630/SX//CNO79/3/IRENA | BD450 | 23.4 |
|  | Resistant check (Urubo or BARI Gom 33) |  | 1.0 |
|  | Susceptible check (Atlax or BARI Gom 26) |  | 83.3 |

* Average values of WB index (%) across experiments are presented.

**Table 8**. Markers significantly associated with wheat blast resistance in 1WBEG and 2WBEG nurseries.

| Algorithm | SNP | Chromosome | Position (Mb) | P value | $R^2$ | Experiment |
|---|---|---|---|---|---|---|
| MLM, MLMM, FarmCPU | Multiple SNPs | 2NS/2AS | 0-35.4 | 1.87E-13 to 9.35E-4 | 0.26-0.50 | All |
| MLMM | IAAV2838 | 1BS | 41.6 | 5.71E-4 to 8.95E-4 | 0.13 | Oki18b, Quir19b |
| FarmCPU | AX-94523488 | 6BS | 51.3 | 2.25E-4 to 2.63E-4 | 0.07 | Oki18a, Quir19b |
| FarmCPU | AX-95215927 | 7BL | 682.9 | 1.84E-7 to 1.26E-4 | 0.35 | Quir18b, Quir19a |

Note: Physical positions for SNPs are from Chinese Spring RefSeq ver. 1.0

**Table 9.** Bi-parental mapping populations for dissecting non-2NS resistance.

| **Female parents (Non-2NS)** | | | **Male parents (Non-2NS)** | |
|---|---|---|---|---|
| **GID** | **Name (Resistant)** | | **GID** | **Name (Susceptible)** |
| 6567313 | PAURAQ/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW// KAUZ/6/PAURAQUE #1 | | 6464471 | KACHU/BECARD// WBLL1*2/BRAMBLING |
| 6682922 | BAJ #1*2//ND643/2*WBLL1 | | 5106391 | SW8488*2/KURUKU |
| 5686539 | CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92/4/BERKUT | | 7890092 | BARI GOM 25 |
| 6564531 | WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/T.DICOCCON/PI94625/AE. SQUA(372)//SHA4/CHIL/5/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1 | | 14337 | ATTILA |
| 6569147 | BAJ #1*2/TINKIO #1 | | 7890088 | BIJOY |
| 27001 | TEPOCA T 89 | | 7890088 | BIJOY |
| 3855011 | VOROBEY | | 14337 | ATTILA |
| 5390612 | SUPER 152 | | 7890088 | BIJOY |
| 6085788 | QUAIU #1 | | 3820759 | KENYA HEROE |

**Table 10.** Crosses made for pyramiding 2NS with non-2NS resistance.

| **Female parents (2NS)** | | | **Male parents (non-2NS)** | |
|---|---|---|---|---|
| **GID** | **Cross (Resistant)** | | **GID** | **Cross (Moderately Resistant)** |
| 7176068 | SUP152/3/INQALAB 91*2/TUKURU//WHEAR | | 6564531 | WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1/4/T.DICOCCON PI94625/ AE.SQUA(372)//SHA4/CHIL/5/WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1 |
| 7806808 | BORLAUG100 F2014 | | 27001 | TEPOCA T 89 |
| 4318735 | MILAN/MUNIA | | 6567313 | PAURAQ/5/KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ/6/PAURAQUE #1 |
| 4754175 | HUIRIVIS | | 6085788 | QUAIU #1 |
| 7890137 | BAW-1260 | | 6682922 | BAJ #1*2//ND643/2*WBLL1 |
| 7895099 | MACS 6478 | | 3855011 | VOROBEY |
| 7895115 | NL1164 | | 7025907 | HD 2888 |

**Table 11.** Quantity of breeder seed produced in the last three years (2018-19 to 2020-21)

| **Wheat variety** | **2018-19** | **2019-20 (kg)** | **2020-21 (kg)** |
|---|---|---|---|
| BARI Gom 33 | 8000 | 26240 | 26265 |
| BARI Gom 30 | 12000 | 12590 | 11870 |
| WMRI Gom 2 | - | - | 1000 |
| WMRI Gom 3 | - | - | 1500 |
| **Total** | **20,000** | **38,830** | **40,635** |

**Table 12.** Quantity of truthfully labelled seed (TLS) of different wheat varieties produced in 2020-21

| **Wheat variety** | **TLS (kg)** |
|---|---|
| BARI Gom 30 | 3300 |
| BARI Gom 33 | 4930 |
| WMRI Gom 2 | 850 |
| WMRI Gom 3 | 950 |
| **Total** | **10030** |

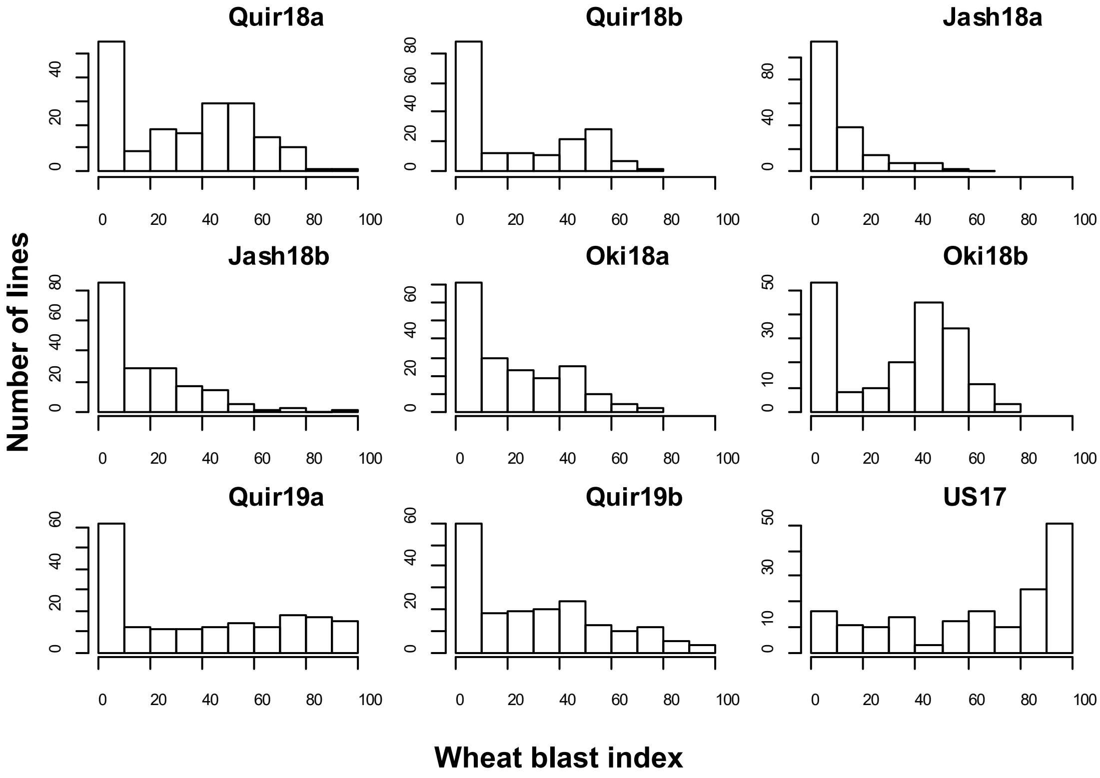

**Fig. 1** Histograms of wheat blast index in individual experiments for 1WBEG and 2WBEG nurseries.

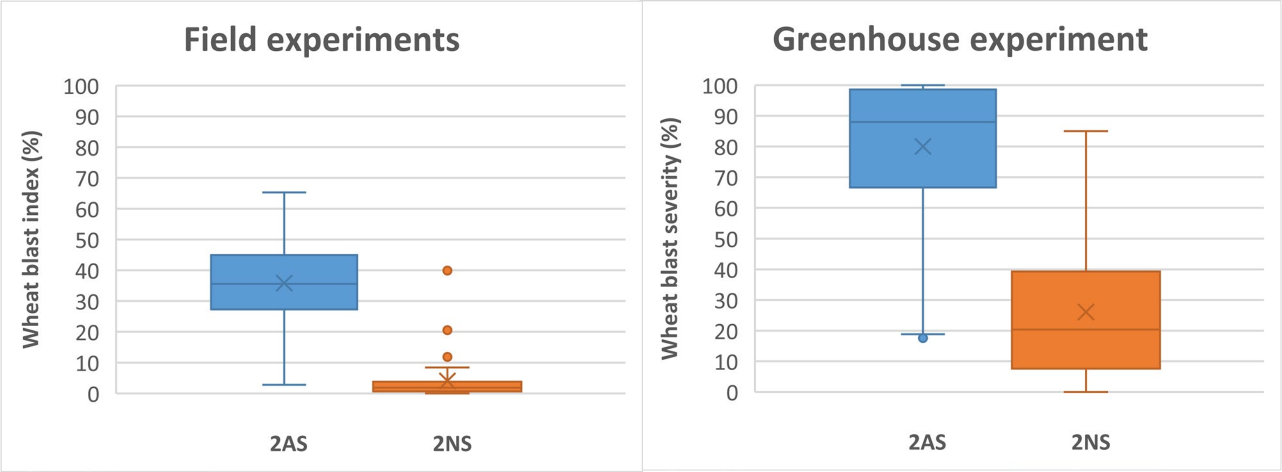

**Fig. 2** Phenotypic effects of the 2NS/2AS translocation on wheat blast resistance in 1WBEG and 2WBEG for field experiments (left) and greenhouse experiment in the USA (right).

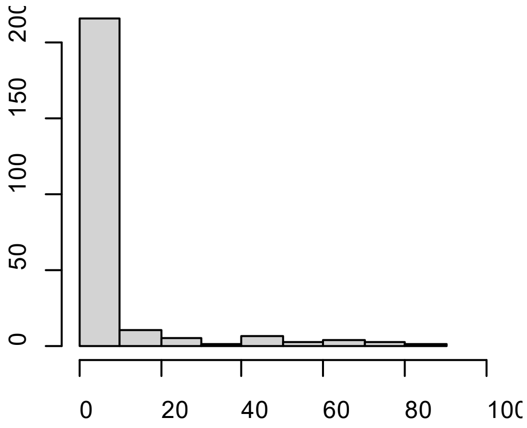

**Fig. 3** Histogram of WB resistance for the HLBSN nurseries

Note: WB data of the five HLBSN were pooled together to show the general trend, despite the fact that they were often evaluated in different experiments.

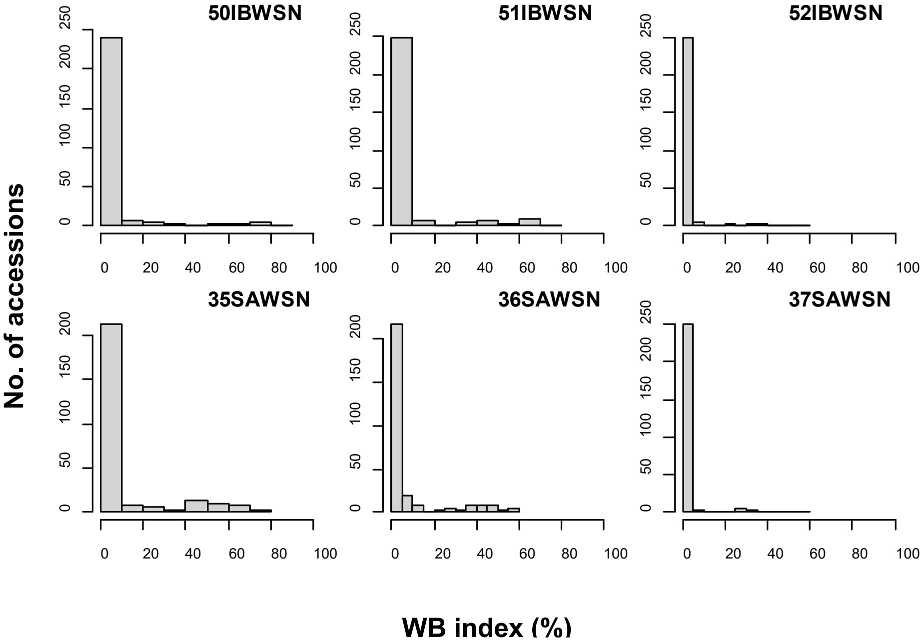

**Fig. 4** Histogram of WB resistance for the six recent IBWSN and SAWSN nurseries

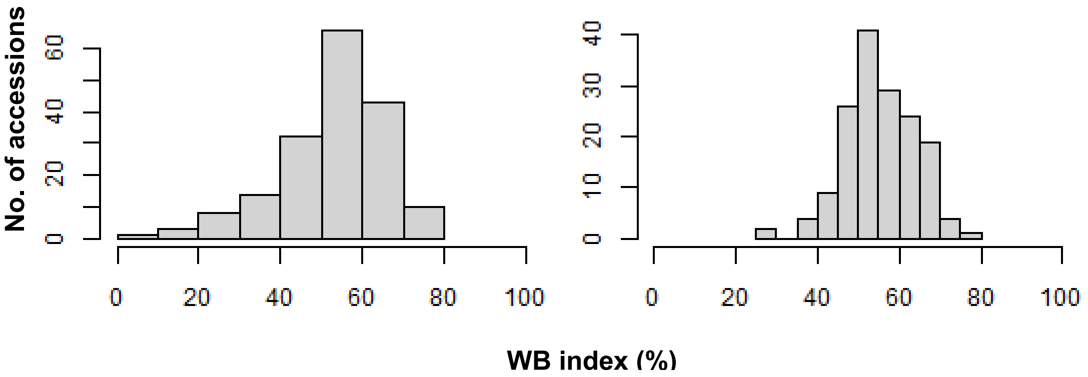

**Fig. 5** Histogram of WB resistance for 50IDSN (left) and 51IDSN (right) nurseries

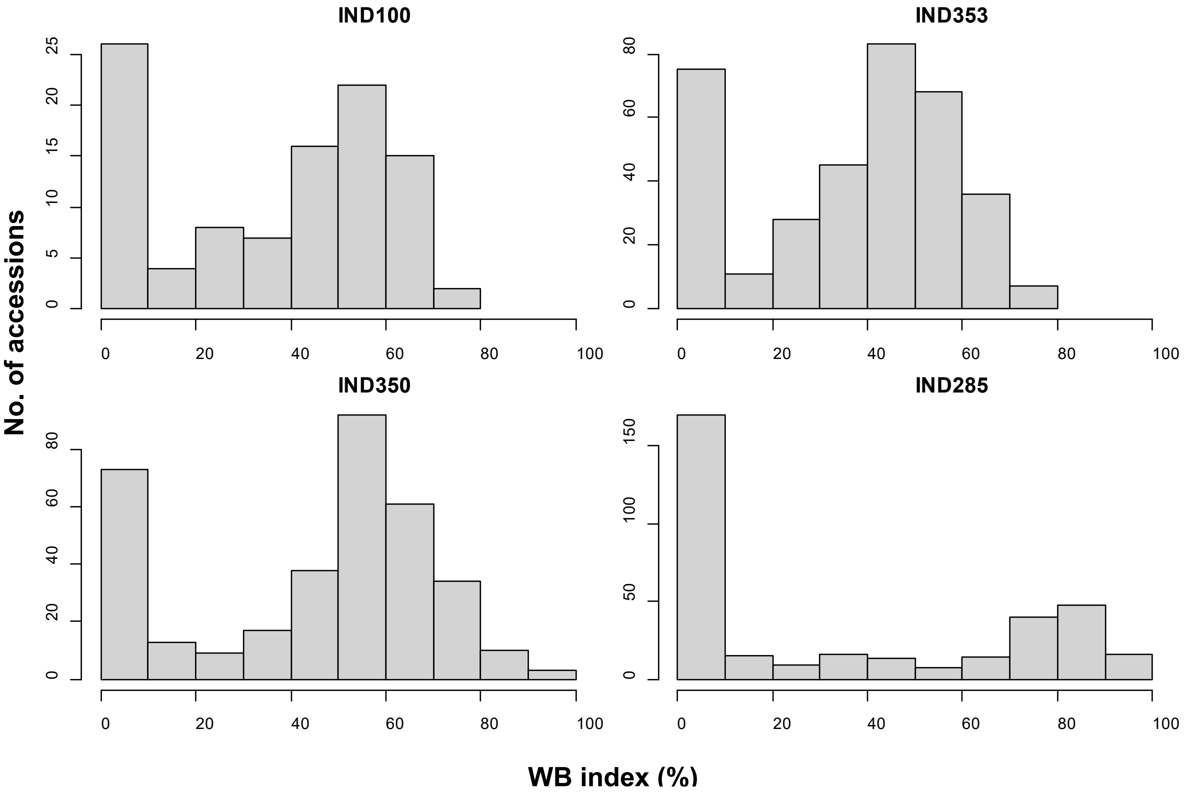

**Fig. 6** Histogram of WB resistance for the four Indian panels

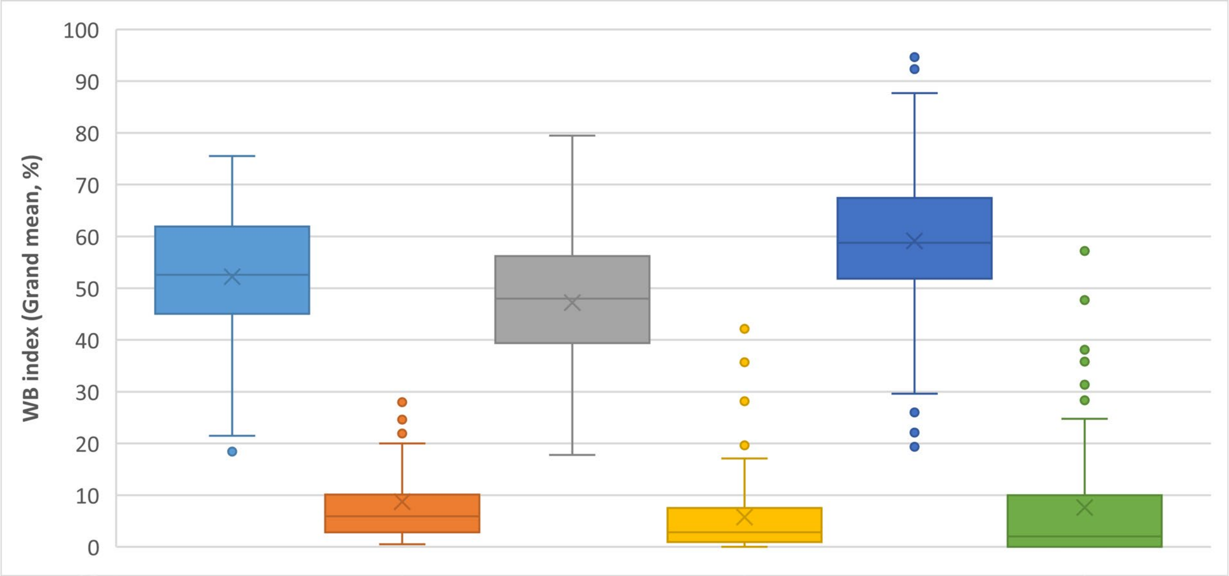

**Fig. 7** Phenotypic effects of 2NS vs. 2AS in three Indian panels. The six groups from left to right are 1) IND100 (non-2NS), 2) IND100 (2NS), 3) IND353 (non-2NS), 4) IND353 (2NS), 5) IND350 (non-2NS), and 6) IND350 (2NS)

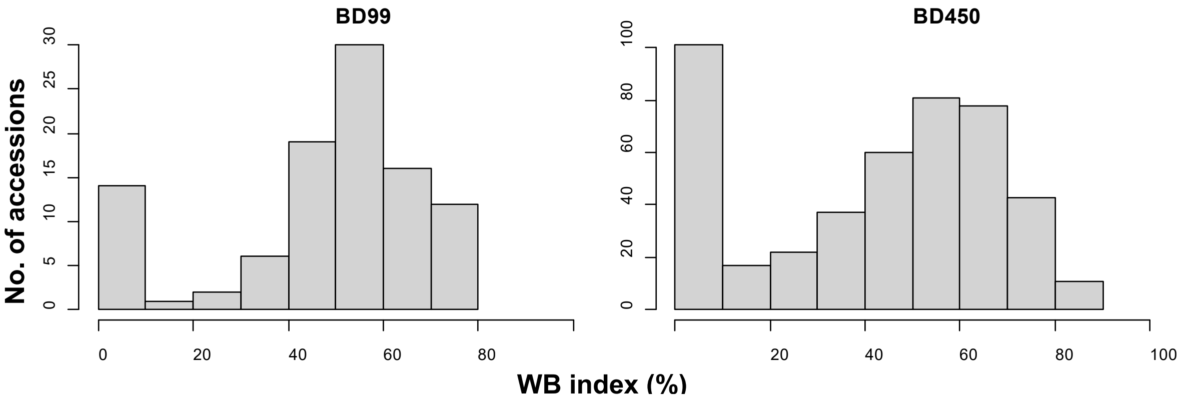

**Fig. 8** Histogram of WB resistance for the two Bangladeshi panels

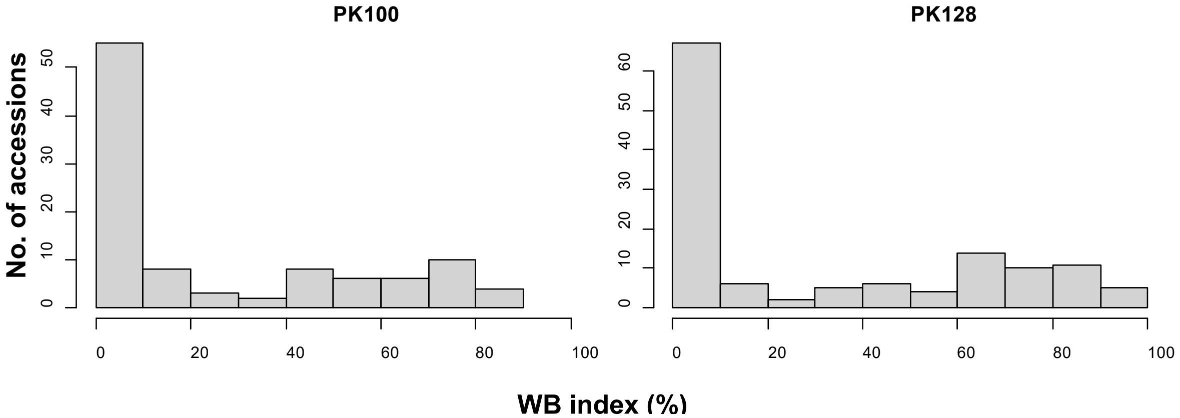

**Fig. 9** Histogram of WB resistance for the two Pakistani panels

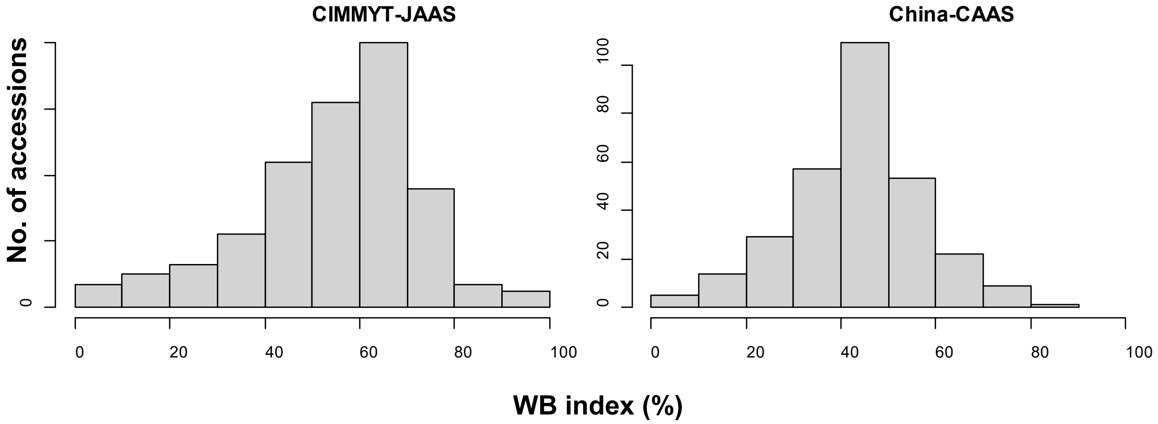

**Fig. 10** Histogram of WB resistance for the two Chinese panels

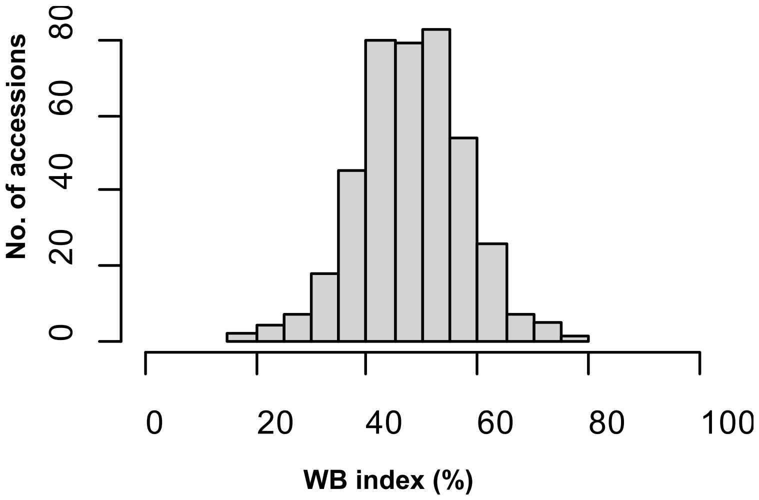

**Fig. 11** Histogram of WB resistance for the Syn423 panel

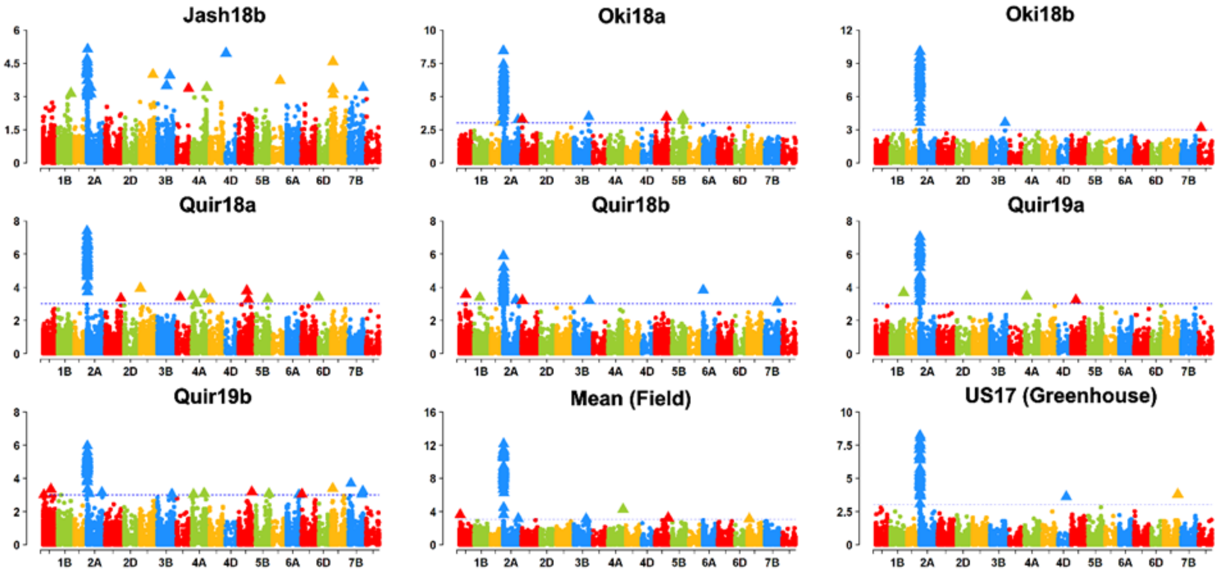

**Fig. 12** Manhattan plots based on MLM model for the GWAS results of 1WBEG and 2WBEG.

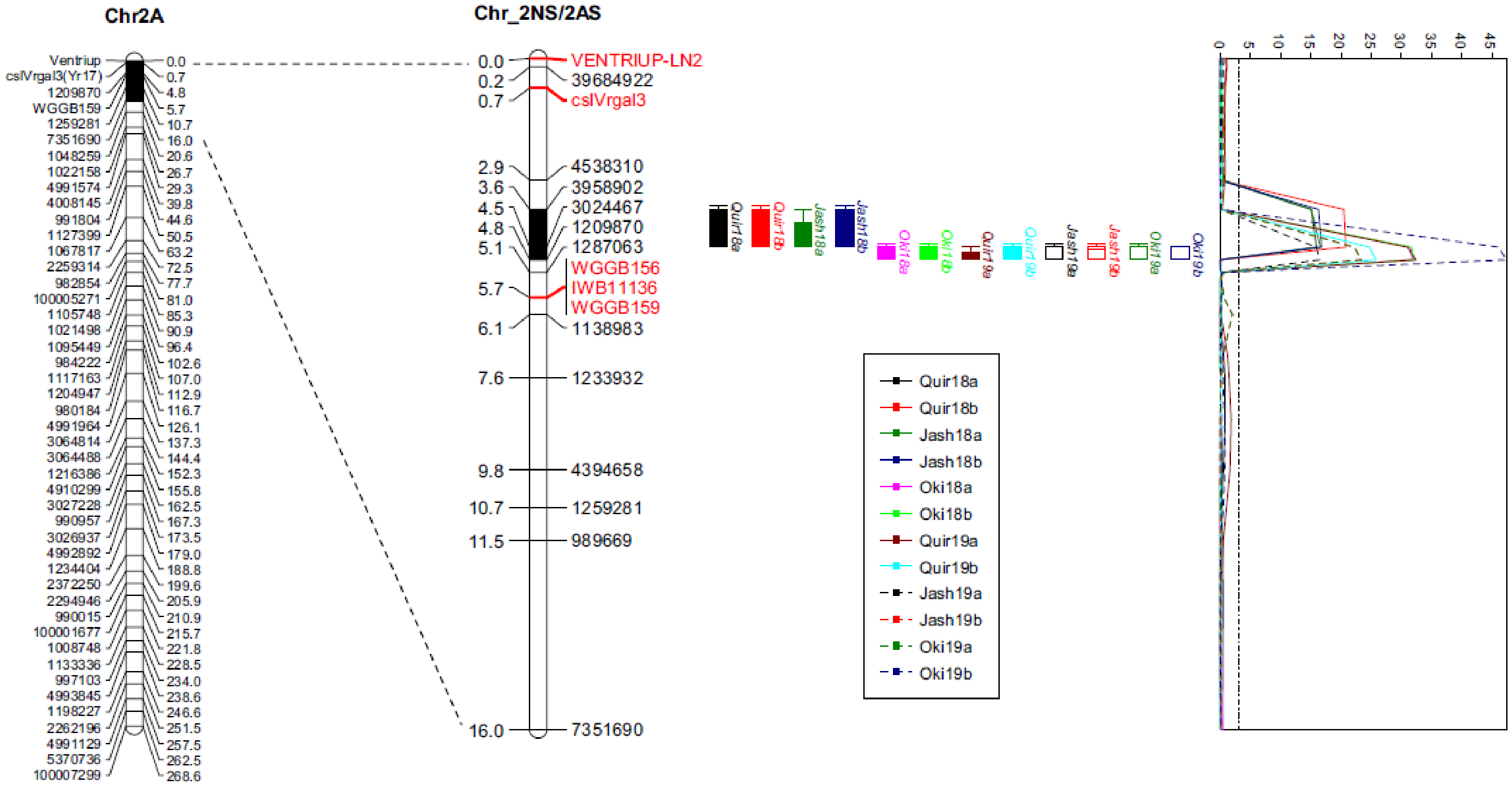

**Fig. 13** QTL profile for wheat blast resistance on chromosome 2NS/2AS across environments in the Caninde#1/Alondra mapping population. The black bar in “Chr2A” delimits the range of the 2NS/2AS translocation, and that in “Chr_2NS/2AS” indicates the QTL region for WB resistance.

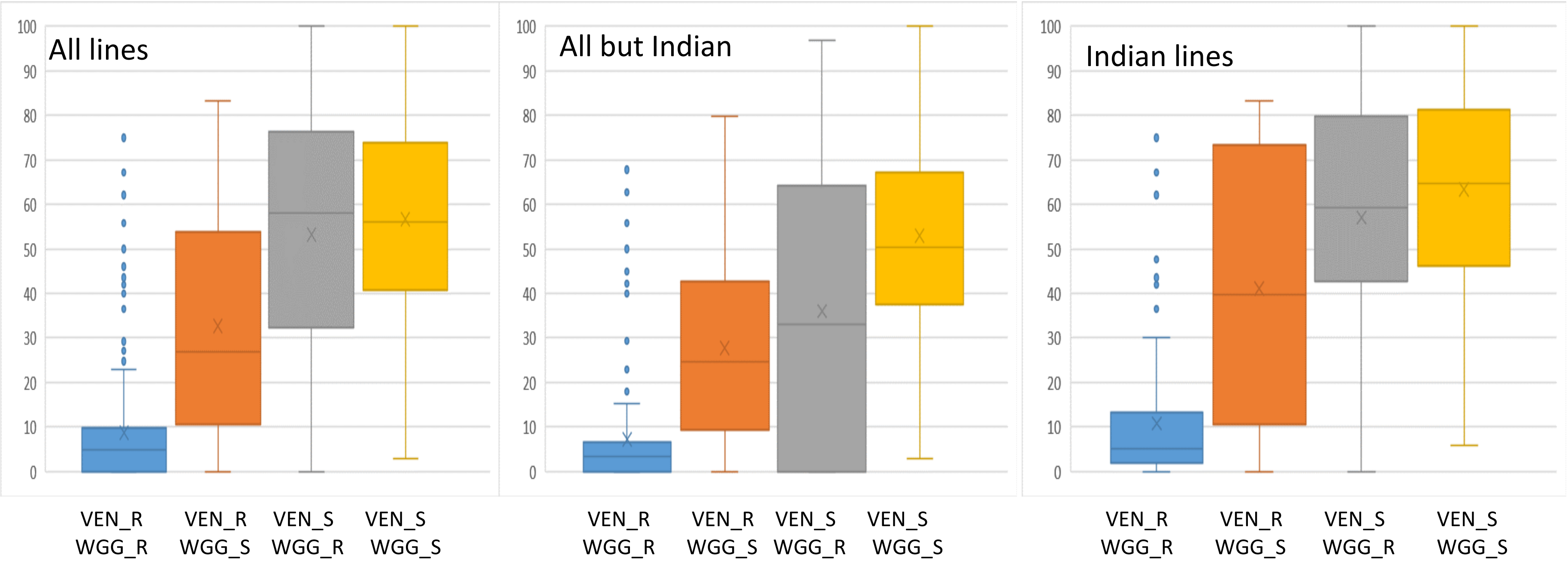

**Fig. 14** Phenotypic effects of different allelic combinations at Ventriup-Ln2 (VEN) and the two STS markers (WGG) in a germplasm collection of 1200 wheat lines. R indicates the presence of the resistance allele, and S the susceptibility allele.