1. Recommendations regarding breeding schemes from meetings in Saskatoon and the expert panel in Mexico, January 2020, need to be followed-up.
   - Realistic options to shorten the cycle time: what biological constraints are there and how can selection intensities be modified to shorten the cycle time?
   - Explore a pipeline where Stage 1 materials are tested in target environments: year 3 split into 2, because Mexico & Africa/Asia testing.
   - If simulations and practicalities suggest more phenotyping in target environments, an adjustment should be made to resource allocation.

Shortening the breeding cycle: the adoption of rapid bulk generation advance (RBGA) saves 1-year from the current 2 generation/year field-based shuttle, allowing a 3-year cycle, as summarized below. We are also piloting a 2-year cycle for Zn mainstreaming which is a modified RBGA scheme (termed rapid cycle recurrent selection; RCRS) using F5 Stage 1 yield testing (2-3 selection environments) for recycling of parents. Both schemes are now being implemented with details summarized below.
Implementation of RBGA/RCRS: Phase I initiation of RBGA and RCRS schemes (December 2020) is based on populations extracted from the existing breeding pipeline to allow optimization of the screenhouse logistics, and the frequency of F4 derived F5 lines/cross with good agronomics, disease resistance & grain characteristics to achieve 10-20 sibs/cross in stage 1 trials (as recommended for improving GEBV). It also enables comparison of germplasm developed from the current shuttle pipeline, RBGA-unselected, RBGA with selection in F4, and RCRS. This includes 100 F2 bread wheat (BW), 100 F2 Zn pipeline and 50 F2 durum wheat (DW) populations for advancement. The F2 BW and DW populations were selected based on breeding values calculated across testing cohorts with grain yield the main criterion along with disease resistance. The F2 Zn pipeline populations were selected based on grain yield, Zn content and disease resistance. The expected outcomes from Phase I are:

- Establishment of generation advancement protocols & logistics (by May 2021)
- Population size and advancement method optimization using actual data for continuous improvement.
- Comparison of grain yield and other traits between current shuttle breeding, RBGA F4:F5-unselected and RBGA F4:F5-selected (estimate genetic gains) and associated cost estimations.
- Trait value comparisons in RCRS and other pipelines.

Phase 2 RBGA and RCRS implementation will commence in May 2021 based on per pipeline crossing blocks with parents selected using GEBVs from current (November 2020-April 2021 Obregon) stage 1 trials. Clustering analysis & selection indices will be used in the parent selection process.

Testing Stage 1 materials in target environments: at present, efforts are focused on the implementation of the RBGA schemes to achieve a 3-year cycle (2-year cycle for Zn pipeline). We are currently developing a data-driven approach to identify representative TPE sites to initiate Stage 1 target environment testing. Based on this we will adjust associated resource allocations.

Breeding scheme simulations

2. Simulation of schemes, including costs, effective population size, time of cycle is key to achieve an evidence-based result and needs to be conducted with EiB and their simulation group.

- Going from 4 to 3 years with constant accuracy is advantageous; tradeoffs need to be simulated, six (current) vs. three (rapid) year cycles.
- Selection indices: need to simulate, including quantitative genetics, using a selection index versus using the shuttle breeding approach.
- The cost of schemes needs to be incorporated into the simulation analysis, running multiple schemes for comparison purposes is prohibitive monetarily.

Accelerated breeding scheme simulations: have now been completed by Fernando Toledo with input from the wheat breeding and EiB teams (see slide deck Annex 1). Simulations used stochastic simulations with historical breeding data in AlphaSimR to compare the current breeding strategy (CBS) with the RBGA and RCRS schemes. The overall trends in the population and in the lines developed (below) clearly show the enhanced genetic gains progress from the accelerated schemes, supported by statistical comparisons (CBS vs RBGA/RCRS <0.001). In addition, the variance trends describe the faster rate of turnover of genetic variance into genetic gain with these schemes (CBS vs RBGA 0.0013; CBS vs RCRS <0.001).
The simulated gains in accelerated schemes are hypothesized to be attributable to greater speed (RCRS) and higher accuracy (RBGA). RCRS & RBGA give similar rates of gains because the greater speed in RCRS balances with the higher accuracy of RBGA. In essence, both speed and accuracy are essential. Based on the requests of the pipeline wheat breeders, the simulations also assessed correlations between traits in the bulks (e.g. the effect of indirect selection for linked or interacting effects). This detected some differences, indicating that correlation between traits (and therefore indirect selection) may have an effect on outcomes. However, all of the correlations assessed included zero in the confidence interval, indicating no substantial effect (in terms of overall genetic gain) on line generation when selection was made in bulks. The use of markers (in context of comparing breeding schemes by simulation) was provided an “insurance policy” for heritability (e.g. recovering missing heritability due to heterogeneous data). Given that marker data is available, it is used for calculating GEBVs for recycling. In future, markers will be used to implement more aggressive GS schemes.

**Selection indices:** are being tested for use in line advancement and parental selection. Initial simulations by Johannes Martini focused on testing selection indices for use in line advancement in the Zn pipeline. This work is now being continued by Philomin Juliana and a summary document is attached (Annex 2). Using data from 2019-2020, an eigen selection index method (with the first eigenvector of the multi-trait heritability used as the vector of co-efficients) shows potential for co-selection of Zn and grain yield (69.6% overlap with breeder selections) and will be tested for line advancement in the Zn pipeline. In addition, selection indices for parent selection will be assessed for each pipeline based on the product profiles and complementing clustering analysis to reduce the dimensionality of the parent selection data relevant to each target TPE. Together, these will provide a data-driven method to allocate elite parental material to the different pipelines and to include selection indices in parent selection and line development.

**Breeding program size and costs:** simulations are underway (March, 2021) to assess crossing schemes followed by breeding program size and comparison of multi-environment testing strategies. Full costings for the breeding programs (bread and durum wheat) have been developed by Leonardo Crespo and Ravi Singh. These are being used to determine genetic gains per unit cost (using the BMGF three-stage modeler) which will allow assessment of costs per unit of genetic gain per breeding pipeline.
3. Speed breeding facility needs to be maximized; optimized operations are required.

- Lee Hickey protocol (U. Queensland, Australia) should be consulted. 30 days to heading is possible.
- Use F3 or F4 derived lines (F2 was discussed but not considered an option). F3 vs F4 will be simulated.

**Speed breeding operations:** Lee Hickey visited Mexico in March 2020 but could not visit Toluca due to the Covid-19 situation which cut his trip short. The protocol being used has been developed in consultation with Lee & the JIC team via ongoing discussion. Suchismita Mondal has responsibility for running the facility and the first round of testing was conducted in August 2020. The initial sowing tested 3 genotypes with distinct heading dates (early, normal and late) comparing two different pot sizes (6 and 8 inch) with 10 reps/treatment (genotype x pot size) under a 20-hour light regime. For the earliest genotype, heading occurred at 30 days and was completed by 42 days in all treatments although some genotypic interactions were detected. On average 3-4 tillers were produced per plant with 15-20 seeds per spike.

The initial testing identified some practical issues with the lighting system which resulted in temperatures above 30C, sterility in the top part of the spikes, production of small spikes, and also highlighted the need for careful maintenance of fertilizer applications. To address these factors and determine if it is possible to perform successful crosses, a second experiment was conducted in September 2020 using different potting mixes, reducing the light regime to 16 hours, and increasing the number of genotypes to six. As previously, heading of the earliest line was at 31 days and spikes were small, with no anther extrusion under light. This resulted in only around 10% of the crosses successfully seeding seed. To further evaluate the issue of crossing, we conducted a third experiment in November 2020 to compare crossing outcomes in speed breeding to another available glasshouse with no supplemental lighting. Results of this testing will help to determine next steps for crossing in the speed facility.

Based on the experiences to date (~6 months of operations) the light and fertilization regimes are established to achieve generation advance in 75-80 days. We are currently testing out operational practicalities to determine maximum capacity (currently operating at 1,800 pots) and maximum number of seeds/pot or trays in order to optimize the space. At present, the protocol is not yet optimized for making crosses, likely due to the poor spike growth and lack of pollen under the light regime. We are looking into the option of using the screenhouse for crossing and are currently conducting crosses in the
screenhouse to evaluate the efficiencies. We will continue to make adjustments and refinements as cycles progress.

**Generation advance:** the issue relating to use of F3- vs F4-derived lines is linked to the time taken for generation advancement within the 3-year RBGA scheme (summarized above) from crossing to obtaining F3-derived F4 matched to the field season in Obregon for Stage 1 trialing. Based on 2021 results it may be possible to go faster in the screenhouse, and if so, F4-derived lines will be used. Alternatively, we will advance early generations in the speed breeding facility.

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**Addressing family structure in trials**

4. The family structure [in yield trials] needs to be addressed, it will have a big impact on how GEBVs are estimated and accuracy.

**Use of sister lines:** is underway in Stage 1 and will be trialed to apply GEBVs to advance lines. The optimum number of sibs needs to be established as there is a trade-off: more sibs/cross versus optimum number of sibs/cross but sibs from more crosses in Stage 1 trials.

Family structure in the yield trial stages have been assessed for different cycles (see below). In Stage 1, the maximum family size was 65 and in Stage 2, it was 44.

Genomic prediction accuracies have been assessed for 10 full-sib families in Stage 1 that had 40-64 full-sibs (number indicated by 'N') to determine the genomic prediction accuracies (PA) when half of the full-sibs were predicted from the other half. The PA values ranged from 0.05 and 0.52. Therefore, it is likely
that different families will have varying prediction accuracies with some well predicted and others less so, making optimization unlikely (as it would be family/cross-dependent). This analysis also shows that the phenotypic differences between sister lines do not have a linear relationship with the genomic relationships between them i.e. highly related full-sibs might have very similar phenotypes, but then there are several full-sibs with a genomic relationship between 0.1 and 0.4 that have a range in their phenotypes and these are the ones that are challenging to predict.

5. Strategic decision analysis must be performed with regards to gene deployment and pyramiding, and cassette development
   - Request that CIMMYT outline a policy statement. APR minor gene concept for Lr, Yr resistance, moving away from single gene reliance on resistance.
   - We need a better understanding of how to deploy and reuse and stack major genes.

Deployment of major genes and adult plant resistances: a document has been prepared by Sridhar Bhavani and Ravi Singh to inform CIMMYT’s ongoing breeding and deployment. The draft is attached as Annex 3 for review and discussion at the April STSC meeting.

TPE-specific breeding pipelines

6. Breeding pipelines must be TPE-specific so that they lead to the development of advanced lines targeted to corresponding TPEs.

Definition and analysis of Indian TPEs: is complete and the associated publication is accepted (Crespo et al. 2021; Annex 4). The overall estimation of grain yield progress (based on analysis of CIMMYT’s international Elite Spring Wheat Yield Trial (ESWYT) trials from 2001 to 2016) shows clear progress for grain yield (below, left panel). To further define progress at a TPE level we used historical meteorological
and soil data, and grain yield information to define three TPEs in India (below, center panel). We estimated their correlated response to selection, and the prediction ability of five selection environments (SE) in Obregon and grain yield (GY) progress in each TPE. The analysis shows different rates of gain in each TPE and that some Obregon selection environments (and statistical models) give better predictions for specific TPEs (below, right panel show results from the best model per TPE/SE combination).

Right panel: Obregon selection environments notated as B5IR (beds, 5 irrigations); B2IR (beds, 2 irrigations), BLHT (beds, late heat), F5IR (flat, 5 irrigations), FDRT (flat, drought). Further description in Crespo et al. (Annex 4).

Follow-on work aims to identify representative sites (reflecting the population of sites within each defined TPE) which could be the basis for Stage 1 testing (or augmented Stage 2 testing), and to develop a TPE-based parental selection strategy (outlined in parental selection below).

**Identifying optimal representative sites for Stage 1 TPE testing:** to change our stage 1 testing strategy from the current use of SEs at Obregon to a scheme that uses fewer SE sites plus representative sites in the TPE (to increase accuracy of Stage 1 testing) we are using Factor Analytic (FA) models to assess GxE contributions per site to select representative sites located in different Indian TPEs using 18 years of ESWYT data. Based on the FA analysis, we can assess the contribution of each site, with an ideal key site (for TPE testing) being one that represents the GxE evenly between factor analytic components (~ 50% FA(1): 50% FA(2)). This also allows definition of stable sites, defined as sites where FA(1) does not represent the GxE and explains most of the GxE. An example of a stable FA(1) site vs. balanced FA(1) and FA(2) contributions is given below. This analysis is complete on a year-by-year basis and the next step is to assess GxE patterns across years using marker and pedigree information to connect entries. This will be presented at the April 2021 AGG-Wheat team meeting.
The full analysis to define optimal testing sites in India will be complete (including feedback from EiB colleagues) before May 2021. We propose it as a data-driven means to classify representative sites within TPEs. Further analysis will link this to the current predictions per Obregon SE. Once complete, we will develop a SOP to inform the testing strategy per pipeline. This will also specify the appropriate check strategy, based on current simulations underway by EiB with input from biometrics colleagues.

**Towards defining and optimizing East African TPEs:** is currently in the planning phase for Ethiopian TPEs, led by Bhoja Basnet and Bekele Abeyo. The initial plan has been presented to the AGG-Wheat team and is being refined before implementation. Briefly, this will encompass analysis of existing data from CIMMYT and partner trials as well as the development of a forward testing strategy. This will use a data-driven approach to identify key test sites for use in yield potential testing and implement a comparative trials series with partners to determine: (1) baseline comparative performance of existing CIMMYT material; (2) predictive ability of Obregon selection environments; (3) heritabilities and GxE components of testing sites; and (4) variance components for forward genetic gains modelling. It will also be used to develop a framework for moving to earlier testing of pipeline material in target environments.

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**Further optimization of breeding/testing**

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**7. GEBV must be routinely incorporated in making selection decisions.**

GEBVs will be used for selection of sister lines to progress from Stage 1 testing and selection of parents for recycling from Stage 1 (see figure on Page 1). Current use of GEBVs for Phase 1 of implementing RBGA is based on multiple cohorts (summarized below) but will be based on a single cohort of Stage 1 testing from the current (2020-21 Obregon) cycle in Phase 2.

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**8. Experimental designs should be optimized**

- Selection and/or managing the environments [optimal vs stressed].
- 1 Rep trial with multiple checks vs sparsely distributed replicated trials.
- Retroactive analysis from maize and rice.
- Obregon: heritability estimates are based on single experiment. What are the heritability estimates across years at Obregon? Can we compare it with TPEs?
- Is there any room for improvement both at Obregon and in TPEs?

**Managed environments:** the move to pipeline-specific selection and more accurate assessment of TPEs will inform the use of specific managed environments. We are developing an approach to weight SE performance in Obregon to (a) select parents per pipeline and (b) predict performance in each pipeline’s TPE. The optimal check strategy to actively track forward genetic gain per pipeline is in development.
(EiB/biometrics). In order to better target TPEs, per pipeline parental selection is now being done using clustering analysis that uses the predictive performance of the selection environments (linked to TPEs). Current progress shows that bi-clustering can be used as a data-driven means to classify elite parents in the crossing block into breeding pipelines, linked to Obregon SEs and TPEs (shown below for Indian TPEs reported in Crespo et al. Annex 4). The next steps are to determine Ne within each bicluster/group of lines for each pipeline and to add layers of information on disease scores and other traits to each bi-cluster. This will inform the use of data from the current Obregon cycle to select parents per pipeline for May crossing in Toluca.

Summary of classification of all lines in Stage 1 testing for crossing block allocation using bi-clustering. This methodology subsets the lines that exhibit similar behavior over a subset of environments. In the figure, the y-axis indicates the SEs and the x-axis indicates the centered and scaled grain yield of the lines in certain SEs. Each sub-plot within the figure represents a bi-cluster. Each bi-cluster (A-O) is conformed for a set of lines that have similar performance over the indicated SEs. Thus, bi-cluster D is conformed by lines that have superior average performance in B2IR, FDRIP and F5IR, which our previous analysis indicate that these SE are correlated to TPE3 in India.

Check strategy: is in development across CG breeding programs under the leadership of EiB. For wheat, BSU colleagues are providing input into the development of an optimal check strategy for each pipeline. This will support the program to move from use of historical analysis of genetic gains across a single program to a forward strategy to calculate realized genetic gain on a per pipeline basis.

Optimal experimental design: some analyses and cross-comparison was done by Johannes Martini using existing data. Consensus is building that SEs in Obregon can be used in developing selection indices or methods of parental selection (as above) for each of the targeted TPEs. Implementation of optimal experimental field designs has not yet been fully achieved due to Covid-19 labor and logistics constraints.
on planting in November 2020 (and therefore could not be applied to the 2020-21 Obregon cycle), although a sparse testing trial was established. The team is committed to making enhancements to experimental design for per pipeline testing for the 2021-22 selection cycle.

9. **All lines in Stage 1 trials should be evaluated in multiple managed environments at Obregon using p-rep or sparse testing designs.**

   - This enables quick identification of superior parents for population improvement as well as the superior lines for advancing into Stage-2 trials.
   - Both selection intensity and accuracy of breeding value estimates can be enhanced by testing lines in multiple environments in Stage-1 trials using designs that enable efficient testing.
   - Logistics for routine integration of markers in breeding, i.e., leaf sample for DNA extraction.

**Managed environments and sparse testing:** all Stage 1 material will be tested in 3-4 managed environments in Obregon (see scheme on Page 1) except for the RCRS pipeline in which seed availability will limit testing to 2-3 environments (due to the 1-year reduction in cycle). The specific choice of managed environment will depend on the per pipeline breeding objective/s. A pilot experiment for sparse testing is being carried out in 2020-2021 growing season. Based on the data analysis from this pilot the optimal design(s) will be implemented in the 2021-22 crop season. Simulations are planned to determine optimal use of sparse testing in TPEs.

10. **Stage-2 trials should focus more on the TPEs than in Obregon.**

    Covered above (Point 6). We propose an initial transition phase with Stage 2 testing in both SEs and TPEs. TPE testing needs building with partner institutions, which require investments in mechanization to improve heritability. We are currently developing data-driven justifications that will enable sound investment cases to lay the foundation for comprehensive Stage 2 TPE testing.

11. **Strengthen the collaboration with national partners, especially in the expansion of TPE-specific stage-2 yield trials.**

    - Provide resources to enhance their trial quality in order to improve heritability estimates.
    - The lower estimates of heritability/repeatability in TPEs might be due to poor trial management which needs to be improved once we begin testing our advanced materials in the TPEs (stage-2).
    - Is earlier testing of Drought or heat tolerant or both type of lines possible in Nepal/TPEs?
    - Do they have enough resources?

**NARS investment:** is strongly supported (for multiple partners in targeted TPEs) and is a core component of AGG and Crops to End Hunger. We have placed an additional team member (Philomin Juliana) at BISA in India to provide more direct support in South Asia. In Ethiopia, Debre Zeit research center (Ethiopian Institute of Agricultural Research) was unable to maintain its experimental and breeder seeds because of the poor condition of the seed store (i.e. poor ventilation, seed damage by weevils, rats, and birds). We identified this as a priority for practical improvement and provided support to renovate the store (see below). This has substantially improved the facilities for maintaining experimental and breeder seeds. Likewise, we have provided funds to centralize provision of crossing and harvest bags to regional partners in order to support NARs breeding.
The work to define TPEs in South Asia (see Annex 4) will be used to target the investment in specific NARS’ trialing capacity. We are working with EiB to define the priority investment needs and to ensure these are linked to target TPEs to maximize future capacity as it is unlikely that the scale of work required would fall within available budget in AGG alone. At the April AGG STSC meeting we are seeking feedback on moving to the metric of achieving genetic gains in farmers’ fields. As a provider of population improvement (and products at present) what is the complementarity that is available or required to support uptake and use by national programs? What investments are needed to translate this capacity into enhanced genetic gain in farmers’ fields?

12. Breeders must be (more) actively involved in managing their research budgets and experiment stations to increase efficiencies.

- The cost of breeding in different pipelines based on product profiles (5 of them, 2 irrigated and 3 rainfed), across different cost items was presented; important traits focused on are yield potential, water-use efficiency, and heat tolerance in the breeding programs.
- Most costs (about 70%) are fixed, with less flexibility to change, and indirect costs go up to 17%.
- Splitting total cost by operations and by pipeline (given overlaps) remains challenging.
- Some cost groupings may be useful – e.g. phenotyping; lab costs. Quality analysis in the breeding pipeline was found to add to the costs significantly; may offer potential if you can predict quality well, and then have more targeted quality analysis.
- Many costs within reach already appear optimized at CIMMYT - other (fixed, IC) costs out of reach.
- Obregon costs appear relatively competitive @ US$ 10/plot; Use line entry costs as basis for costing
- Some costs evolve – e.g. advances in genome sequencing and lower costs

**Program costings:** have been made with the next step to look at the variable costs per pipeline, led by the pipeline breeders (Leo Crespo, Bhoja Basnet and Velu Govindan) and Suchismita Mondal (accelerated breeding and trait pipeline). This is underway via the BMGF Genetic Gains Modeler which is being customized to the wheat pipelines with EiB and BSU support. Whilst this is underway, the cost per unit gain is being approximated using the simulation results. Each of the four breeders have been assigned an operational budget to ensure that they can adjust variable parts of their pipeline for optimization whilst maintaining centralized support functions that ensure smooth running of the whole program.

13. **Efficiency can be increased through carefully designing experiments, influencing costs significantly.**

The challenge lies in that most breeding costs are fixed and reducing costs to increase efficiencies is complicated. Every breeding program has its own challenges, which are specific to the location of trials. Developing general
guidelines for managing a dynamic breeding pipeline is not feasible, however, despite these constraints, opportunities to increase efficiencies include:

- Using breeding tools (genomic selection; database);
- Costing on line basis - not per unit (e.g. to assess entries at YT - 2 reps x 1 environment; vs many environments; genotyping implies 1-off line cost);
- Take lessons from 2 past BPAT (Breeding Program Assessment Tool) evaluations – but overall already efficient and inexpensive phenotype;
- Land charges are relatively expensive; assess potential savings in terms of where operations/trials are best located;
- Look into learnings from EiB and use cost info to optimize.

**Efficiency savings:** existing investment in accelerated breeding infrastructure (speed breeding and screenhouse) enable implementation of 3-year breeding cycle. Other possible efficiency savings are important and as above (point 12), costing analysis is underway on a per pipeline basis. This will also inform the forward pipeline investment cases and the allocation of variable costs to breeding activities. We are adopting EBS for use as a breeding management system, and already use GOBii to store and use genotyping data (due for integration into EBS). We are also in Phase 2 of the design process for shared service mid-density genotyping for use in routine genomic selection.

14. **Identify and use available technologies (e.g. GPS and satellite data) and existing data sets to increase accuracy of data and free resources to invest in priorities.**

Investment opportunities include:

- **Info/data management:** there have been improvements, but still a bottle neck and room for improvement;
- **Equipment replacement,** including technology improvement; genotyping capability; robotics; automating quality testing; data capture;
- **Increasing efficiency of data collection** by NARS/partners – including (re-training); basic equipment.

**Technology adoption:** is a priority with a current core focus on the adoption and implementation of EBS for breeding data management. The wheat program is an early adopter and tester of EBS and has now progressed to the upload of field plans and information for the 2020 Obregon cycle. The next phase of EBS development will look at the analytics capability and we are engaging with the EBS team to ensure this functionality is optimized. In terms of available remote sensing technologies, the current analysis of TPEs (see Annex 4) uses extensive meteorological and soil variables for environmental characterization. We are currently in discussion with Gustavo Teixeira (EiB) and the wheat physiology team (Matthew Reynolds and Francisco Piñera) to use remote sensing approaches to augment the current TPE characterization, building on work already underway in South Asia as part of the KSU-led Feed the Future project.

15. **Consider including straw/biomass as a release (product profile) criterion – it is an important acceptance trait for most (crop-livestock) farmers.**

**Potential for straw/biomass products:** yield and biomass have the highest correlation among any of the yield components. To maximize biomass production, it is necessary to strike the right balance between maturity and plant height without affecting lodging. Therefore, straw yield is almost optimized in elite lines. **Current Crops to End Hunger market segment analysis (underway with EiB) should help to quantify if creating an additional market segment for this specific demand should be a priority. If so, a criterion can be developed.**