Durum wheat breeding manual 2020-2025

A product profile strategy

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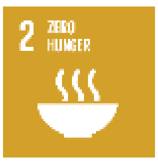

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Acronym list

ACSAD	Arab Center for the Studies of Arid zones and Dry lands
ADYT	advanced durum yield trials (Stage 2)
APR	Adult Plant Resistance
AWAI	AMMI Wide Adaptation Index
BMS	breeding management system
CIMMYT	The International Maize and Wheat Improvement Center
CB	crossing block
CRP	CGIAR Research Program
CWANA	Central and West Asia and North Africa
CWR	crop wild relatives
EU	European Union
GY	grain yield
ICARDA	The International Center for Agricultural Research in the Dry Areas
IDON	international durum observation nursery
IDYT	international durum yield trials
IFWDON	international facultative winter durum observation nursery
INRA	Institut national de la recherche agronomique
ISRA	Institut Sénégalais de Recherches Agricoles
GGE	genotype + genotype x environment
GluInd	gluten index
GS	genomic selection
MAF	Minor allele frequency
MAS	Marker Assisted Selection
Mixo	Mixograph
NARS	National Agricultural Research System
NIR	Near Infrared Reflection
PDYT	preliminary durum yield trial (Stage 1)
PPs	product profiles
RDA	recommended daily allowance
SDGs	Sustainable Development Goals
SDS	gluten strength by sodium dodecyl sulfate
SSD	single seed descent
SNPs	single nucleotide polymorphisms
TKW	1,000-kernels weight
TP	training populations
TPE	targeted population of environments
USDA	United States Department of Agriculture

A product profile strategy for durum breeding

A product profile breeding strategy aims to: i) identify the main competitors (existing cultivars), their advantages and limitations, and the use of these to define the additional traits needed to replace them in the market; ii) reduce breeding time, costs, and efforts to deliver the best possible new cultivar; and iii) determine the true breeding targets (clients and agro-ecologies) and use them to Drive the selection strategy.

The durum wheat breeding program of the International Center for Agricultural Research in the Dry Areas (ICARDA) aims to deliver new varieties that respond to the Sustainable Development Goals (SDGs) of SDG1: no poverty; and SDG2: zero hunger across several developing countries, with a focus on non-tropical Drylands. Within these goals, the specific product profiles (PPs) are identified in interaction with the National Agricultural Research System (NARS) partners. Each PP corresponds to a targeted population of environments (TPE), which is identified by means of a principal component analysis (GGE) of Stage 4 yield trials combined with climatic and social characterizations. Each PP is also assigned a weighted selection index to effectively select for the most critical traits. In the Stage 2, 3 and 4 yield trials, each entry is defined as matching a given PP when it shows high yield in the station that better represent the TPE of that PP, and a high score for the related selection index. An elite is deemed ready to advance to Stage 5 when addition of the two scores also matches the required phenology and height characteristics.

The overall breeding strategy is to design targeted crosses and advance generations rapidly to $F_{1:4}$ by means of selected bulks and shuttle breeding to avoid imposing stringent selections outside the TPE. The F_4 are then assessed across TPE and advanced via pedigree to F_5 in the off-season. Stage 1 yield testing is also conducted outside the TPE due to logistic considerations, and only traits that are common across PPs are selected. Stage 2 is conducted across TPE to evaluate stability (GxE), a critical trait for climatic adaptation, and performance in the specific TPE. Stage 3 are tested directly at the target environment of each PP by NARS partners. Stage 4 are selected via Stage 3 results, and equally tested by NARS partners.

The general strategy for population enrichment aims to hybridize Stage 2, 3, and 4 entries with good matching (weighted index) to one or more PP. In addition, 2/5 of the crosses are made between the best Stage 4 entries and foreign germplasm coming from other breeding programs, pre-breeding efforts, and landraces. Wide-crosses are integrated at the F_2 level via selection of germplasm produced by the genebank between Stage 4 and wild relatives. A step of recurrent genomic sequencing (GS) is incorporated at the F_4 level for the most promising crosses.

Two parallel breeding programs are conducted in Lebanon (Terbol) and Morocco (Marchouch) to target West-Asian and North African TPE, respectively. In addition, a facultative winter program is conducted in Lebanon (Kfardan) and Morocco (Annoceur) for specific PPs. A further three breeding programs for selection among $F_{1:5}$ are run by NARS partners in Ethiopia (Debre Zeit), India (Amalaha) and Senegal (Fanaye).

In total, six durum wheat breeding programs exist within ICARDA to serve eight mega PPs.

This document provides the reasoning, practical, and logistic details on how to convert this strategy into practice.

- Competitors: several cultivars, depending on the country. Each one is used as trial check in Stage 3 and Stage 4 trials.

- Clients: national breeders. Each is asked to fill PP agreements and define the traits of interest and the competitor cultivar.

- Agro-ecologies: several environmental and social differences. TPE are defined using Stage 4 international yield data (IDYT).

- Traits definition: weighted selection indexes are defined based on web surveys with national breeders and integrating farmers and socio-economic analysis.

- Breeding time: selected bulk shuttle breeding with F_4 multi-location selection and satellite $F_{1:5}$ breeding programs are used to reduce breeding time, maximize heterogeneity, and target the maximum number of TPE.

- Breeding cost: Stage 1 costs are kept as small as possible by avoiding the pedigree method. The costs for each stage need to be computed and used to Drive decisions and investments.

A history of durum wheat

Durum wheat (*Triticum durum* Desf.), whose ancestor was wild emmer (*Triticum dicoccoides*, AABB, n=14) originated from a naturally occurring cross between *Triticum urartu* (AA, n=14) and an *Aegilops speltoides* (GG, n=14) subspecies that has not yet been identified. This wild emmer was then domesticated by ancient farmers to become emmer wheat some 16,000 years ago (*T. dicoccum,* AABB, n=14). For over 4,000 years, this primitive crop was cultivated and traded. Around 10,000 years ago, the Levantine farmers further domesticated emmer to become durum wheat (*T. durum* Desf., AABB, n=14). From here, the Phoenicia traded it all over the Mediterranean coasts to make this crop one of the fundamental staples of the whole Mediterranean diet. However, approximately 5,000 years later, Ethiopian farmers also domesticated emmer into a different subspecies of durum wheat defined as *Triticum durum sub. Aethiopicum or Abissinicum* (Fig 1). From a breeding perspective, this double origin is of great importance as it reveals that ancient germplasm from different geographies can provide great genetic diversity which can be exploited. In addition to the Ethiopian type, the North African and Central Asian germplasms have accumulated the most variations and are therefore specifically targeted in the ICARDA breeding program.

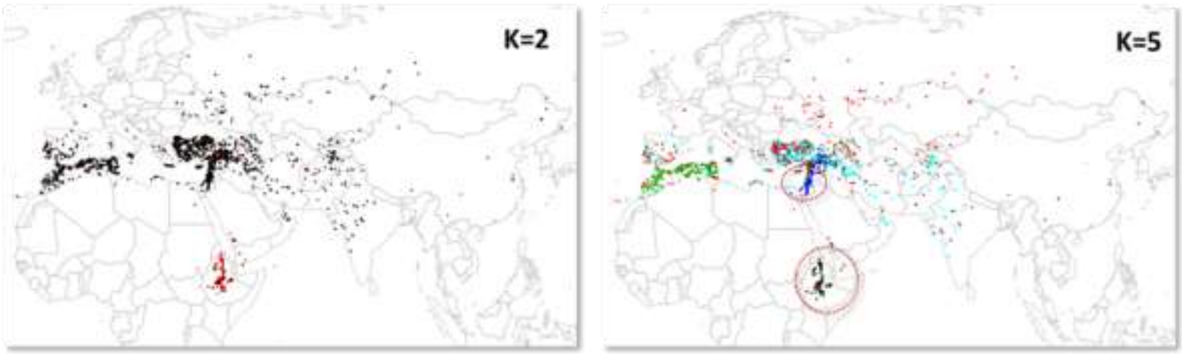


Figure 1. Diversity analysis for two K levels (two and five) of durum wheat landraces

The different 'K' clusters are color coded. It is possible to see how Ethiopia and the Levantine are two different centers of origin, while North Africa and Central Asia appear as two centers of diversity.

In 1920, an Italian breeder Nazareno Strampelli re-discovered the principles of Mendelian genetics and was among the first to apply these to breeding. The first variety ever to be registered in cereals was developed by Strampelli under the name Senatore Capelli, which was derived from the mass selection of a Sicilian landrace population known as Bidi, which possibly originated from North Africa. Since then, Italian breeders have dedicated significant resources to the identification of new superior varieties of durum wheat, including the single most widely grown durum variety ever cultivated: Simeto.

Since then, durum wheat breeding programs have developed all over the world, including at ICARDA and the International Maize and Wheat Improvement Center (CIMMYT). Today, some of the most important impact in terms of durum wheat germplasm can be attributed to the international CGIAR centers, which have contributed to the vast majority of durum wheat varieties, rather as direct releases such as Waha (syn. Cham 1) and Yavarros (syn. Karim), or by their use in the crossing program.

Importance of durum wheat cultivation

Durum wheat is considered a staple food in the Mediterranean diet in the form of bulgur (concassed grains), couscous (also known as *dalia* in India), pasta and unleavened breads, and its straw is very important as animal feed. However, bulgur, couscous and pasta are now also produced on an industrial scale and, in 2016, the market value of the pasta industry alone was estimated at US$11 billion, with an expansion trend of +20% in the next 5 years. The same industry relies on durum wheat to produce high quality semolina. The average price of durum wheat is typically 15-20% higher than that of bread wheat (Fig 2). A premium price of 10-20% over the basic price is paid in developed countries for grains with good color (yellow pigment), large grain size (hectoliter weight), and especially when protein content is above 13%. Consequently, durum wheat has also become a major cash crop that can provide good returns if the right varieties and optimal agronomic practices are used. It is interesting to note how prices have remained high in North Africa, while global prices have Dropped in the recent years. Some raising trends can be seen in Q3 of 2020 due to a combination of droughts and the coronavirus pandemic.

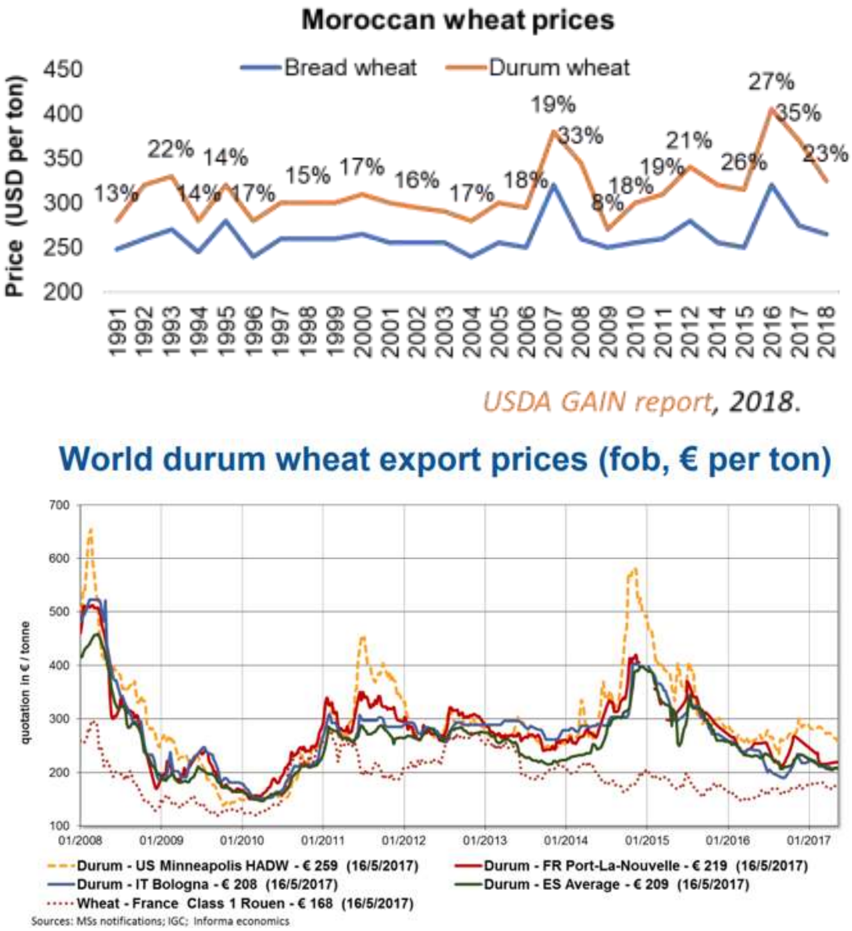
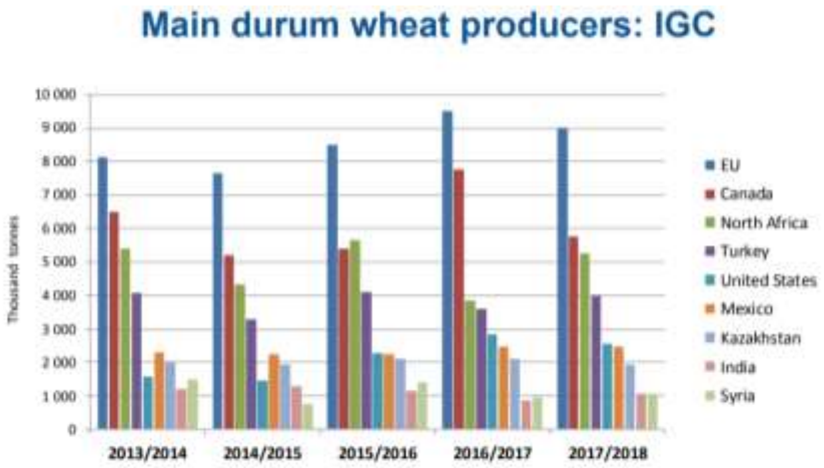


Figure 2. Moroccan and global annual average prices for durum and bread wheat
Source: Adapted from the United States Department of Agriculture (USDA) and Global Alliance for Improved Nutrition (GAIN) report, 2018.

In the past, the European Union (EU) was the main producer of durum wheat and remains a major player. However, in 2017, Europe produced only 9 million tons of the 40 million produced globally (22%). In recent years, Canada, North Africa and Turkey have become the main players in global production, together with Kazakhstan, Mexico and the United States, and also India (Fig 3).



Million tons	2014/15 estimated	2015/16 forecast	Million tons	2014/15 estimated	2015/16 forecast
World total	**32.6**	**36.1**	Argentina	0.3	0.3
EU	7.1	7.5	Syria	0.8	1.4
France	*1.5*	*1.8*	Turkey	2.1	2.4
Greece	*0.8*	*0.7*	India	1.3	1.2
Italy	*3.7*	*3.9*	Algeria	1.3	2.5
Spain	*0.8*	*0.9*	Libya	0.1	0.1
Kazakhstan	2.0	2.1	Morocco	1.4	2.3
Canada	5.2	4.8	Tunisia	1.3	1.3
Mexico	2.3	2.3	Australia	0.5	0.5
USA	1.4	2.1	Others	5.7	5.5
Fonte: International Grain Council					

Figure 3. Durum wheat production (tons) of main international players
Source: International Grain Council

The role of these countries in durum wheat production becomes even more evident when analyzing the cultivation area. In 2017, Italy cultivated 1.4 million ha, Spain and France 0.4 million ha each, and Greece 0.3 million ha (Fig 4). In the same year, Canada planted 2.1 million ha, Algeria and India exceeded 1.4 million ha each, Morocco, Syria and Turkey, between 1.0 and 1.2 million ha, and Azerbaijan, Ethiopia, Iran, Kazakhstan, Pakistan and Tunisia cultivated between 0.3-0.5 million ha. In essence, the EU, and Italy specifically, is no longer associated with the largest area for durum wheat cultivation. This means that new markets have become of interest for seed and breeding companies. While Australia, Canada, France, Kazakhstan, Mexico and Turkey represent the bulk of exporters, North Africa and Italy remain the main importers. The only new player in the import market has become Côte d’Ivoire, where pasta is produced for export across Africa. China has not yet entered the game, but for how long?

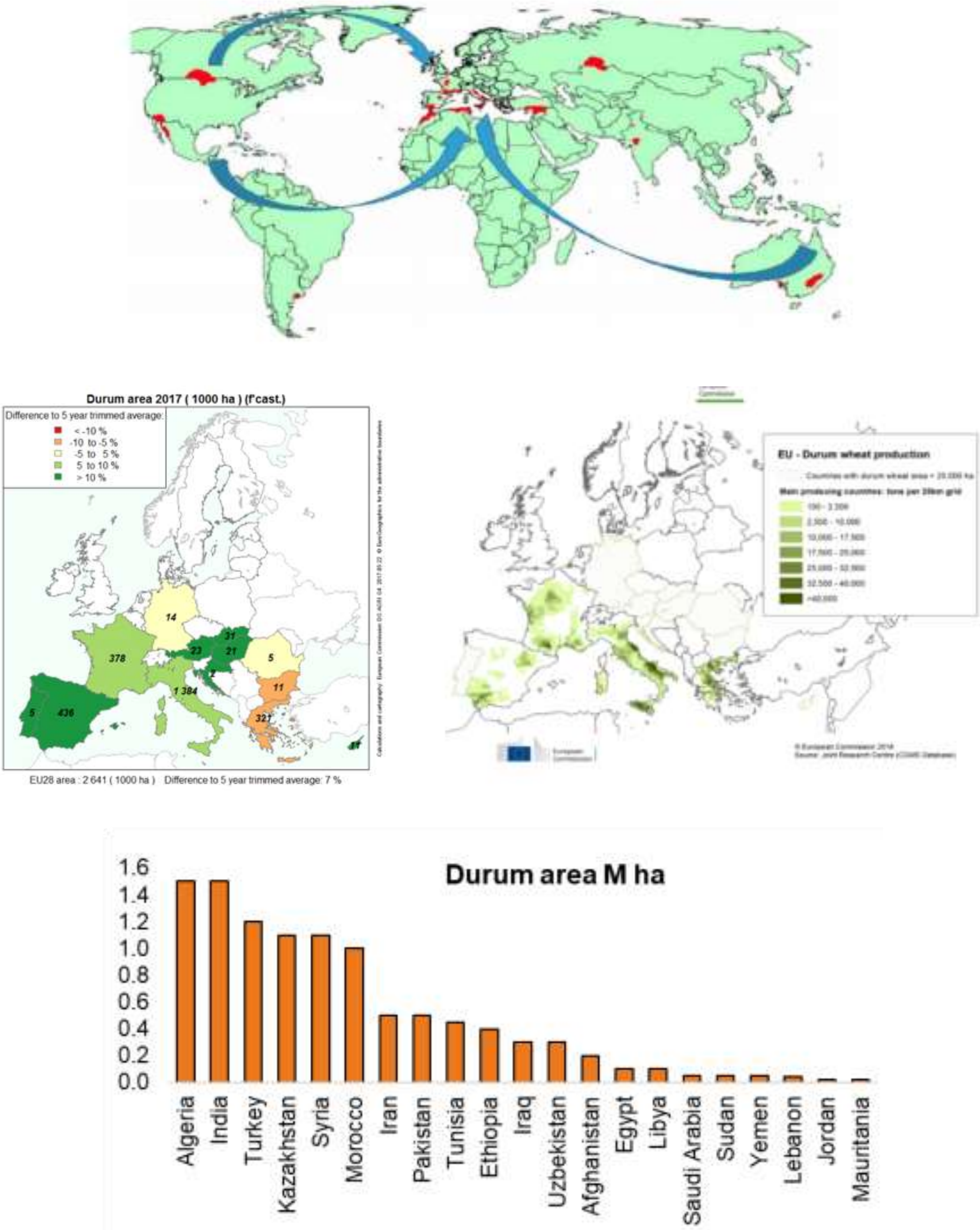


Figure 4. Global durum wheat production in surface area
Adapted from various sources, the first map shows the production areas in red and arrows indicate export trends.

History of the durum wheat program at ICARDA

Durum wheat breeding activities began in 1977 in Lebanon when ICARDA was established. The Center was then moved to the Tel Hadya station in Syria. Until 2003, the durum breeder position at ICARDA was provided by CIMMYT, thereafter it was reassigned as an ICARDA position. In 2005, an initial attempt was made to reintegrate ICARDA and CIMMYT under one wheat improvement program. In 2012, this attempt was boosted by the creation of the CGIAR Research Program (CRP) WHEAT. In 2015, under the title of One Global Wheat Program, the two centers agreed to work together again, with ICARDA focusing on rainfed agriculture and Drylands and the global leadership in durum wheat, since most production occurs in the Center's region of operations.

The initial breeding activities of ICARDA were to test and finalize the selection among F_5 populations developed by CIMMYT. This effort resulted in the first release in 1984 of Waha in Algeria, a cultivar that still occupies more than one-third of the cultivated area. The first independent cross was made at the Tel Hadya station in 1980 between the elite line Jori and the landrace Haurani. This resulted in the release of the cultivar Omrabi1 in Morocco in 1989, which still occupies a good portion of the most drought-prone regions of the country. Over the years, the ICARDA durum breeding program was able to release 125 confirmed varieties in 22 countries (Fig 5). The largest documented impact occurred in Syria, where the work of ICARDA allowed the country to change from a durum wheat importer to a net exporter until the time of the civil unrest in 2012. A total of 11 varieties were released in Syria and their adoption has been extremely fast and impactful (Fig 6).

From 1982 until 2014 (30 years), the durum breeding program was led by Dr. Miloudi M Nachit. In 2012, the program was moved out of Syria to be re-started in Morocco. Historically, the stations of Tel Hadya and Terbol (Lebanon) hosted the program, but since the move to Morocco, Marchouch and Terbol became the primary stations. Dr. Nachit used Tel Hadya and Terbol to deploy his method defined as "double-gradient selection", which used different planting dates and irrigation amounts to substantially create six environments. Additionally, he used international nurseries to access stations across several countries in partnership with National Agricultural research Systems (NARS) to create multi-environment testing. Dr. Nachit also pioneered the use of molecular markers in breeding, and published a paper on RFLP (restriction fragment length polymorphism) in durum wheat dated 1996. In 2009, he was knighted by the King of Morocco for his contribution in protecting Moroccan farmers from the threat of the Hessian fly (together with other Moroccan scientists). One of Dr. Nachit's main visions has always been the use of wild relatives and landraces in durum wheat breeding, resulting in the release of many varieties derived from these wide crosses.

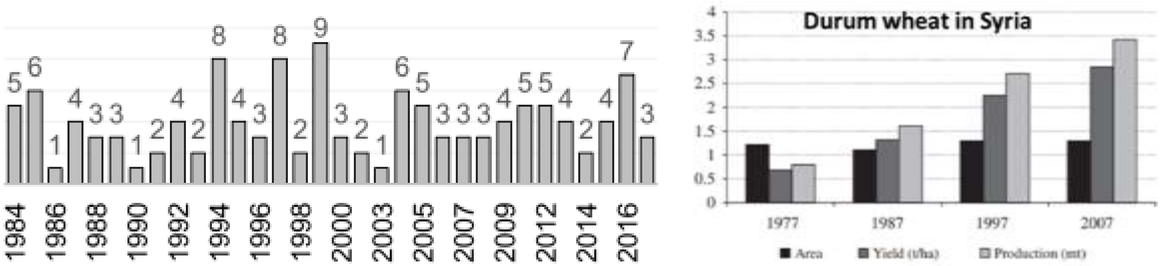


Figure 5. Confirmed variety released over the years and effect of the ICARDA breeding on durum wheat productivity in Syria

Left- showing the number of ICARDA-derived durum wheat varieties released over the years. Right – production area and yield of durum wheat in Syria since the establishment of ICARDA in 1977.

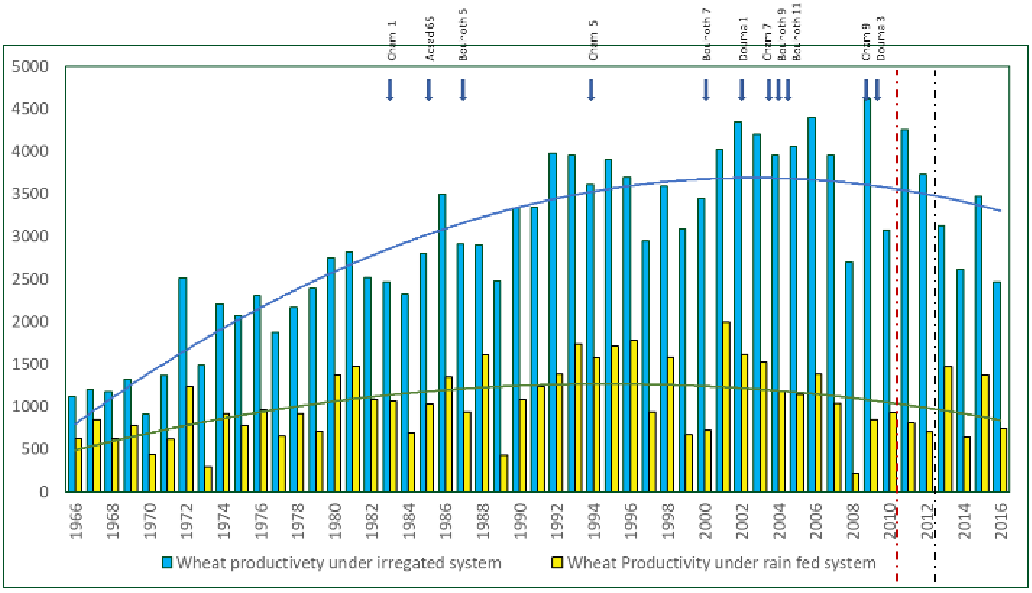


Figure 6. Impact of released durum wheat varieties on Syrian productivity under irrigated and rainfed conditions expressed as yield (kg ha^{-1})

The red vertical dashed line indicates the beginning of the Arab Spring, whilst the black vertical dashed line indicates the beginning of the civil war.

In 2013, Dr. Filippo M Bassi joined Dr. Nachit's team as an associate breeder, and, in July 2014, he took over the program as durum wheat breeder. This manual reviews changes made to the program under Dr. Bassi's leadership, some of which are listed below:

- Strengthening of the International Nursery service by reducing the number of entries and providing more information.
- Shifting from double-gradient to actual multi-locations selection, incorporating a strong component of stability analysis (GxE).
- Incorporation of early generation multi-locations selection.
- Accelerated turnaround to reach Stage 1 testing in just 4 years instead of 6.
- Continued and increased use of crop wild relatives in the program.
- More intensive integration of molecular markers in breeding.
- Development and deployment of PP breeding.

Figure 7. Dr. Benbelkacem, Dr. Nachit, and Dr. Bassi in Beni Mestina, Algeria, in May 2015

Table 1. Brief summary of the program history since it moved from Syria

Year	Goals	Achievements
2013	Re-establish test sites after moving out of Syria	Trials planted across sites in Morocco and Lebanon and combined with international yield data (GGE model)
2014	1. Better integration of experimental designs 2. Characterization of germplasm 3. Improve services of International Nurseries (Stage 3 and 4) 4. Selection of best germplasm from pre-breeding populations	1. Dropped RCBD and all un-replicated trials in favor of alpha lattice and augmented design; increased plot size to 7.5 m^2 to absorb more error 2. Initiated several PhD programs to characterize germplasm for many traits 3. Reduction of International Nurseries number of entries but with stronger selection pressure
2015	1. Incorporation of most recent climatic models into the breeding program 2. De-prioritization of minor diseases 3. Re-evaluation of test sites 4. Better integration in the durum community	1. In discussion with three climatologists: initiate across locations selection when more variability is available (F_4) 2. Abandon Russian wheat aphid, and stem saw-fly, de-prioritize *Septoria,* and no more FHB work 3. Conducted GGE model of Stage 2 vs Stage four trial sites: two useful sites needed improvement; one was replaced
2016	1. Initiation of a small GS pipeline 2. Integration of G + GxE model in Stage 3 to 4 selection 3. First attempt at defining PPs at Stage 4 4. Identification of best-bet traits for drought and heat	1. 20 families put under GS. Publication of an idea paper. 2. Use of AMMI Wide Adaptation Index (AWAI) in selection. Publication of AWAI use on the cover of *Crop Science* journal 3. To align with ICARDA's strategy and development outcomes, the Stage 4 elites were assigned to SDGs as reducing

		poverty reduction or increasing food security, based on the set of traits and their use in specific farm systems 4. Completion of PhD students' phenotypic research: grain size and root depth for drought, spike fertility for heat. Started recording these in all Stage 2 selections
2017	1. Establishment of a disease screening nursery 2. Validation and integration of markers for Marker Assisted Selection (MAS) 3. Integration of industrial quality selection 4. Integration of G + GxE models in Stage 2 to Stage 3 selection 5. Integration of PPs in Stage 3 to Stage 4 selection 6. Confirmation of value in using wide crosses 7. Establishment of satellite breeding activities in Ethiopia, India and Iran	1. Training of staff and equipment to do artificial inoculation in the off-season in Lebanon and in garden tests in Morocco 2. Best-bet traits converted and validated to KASP. Use at Stage 2 selection 3. Complete profile of Stage 2, Stage 3, and Stage 4 lines 4. Inclusion of these models in Stage 2 5. Using Lantican et al., 2016 survey, definition of a weighted selection index for PP and use of this to select Stage 3 to Stage 4 6. Release of three varieties derived from crop wild relatives (CWR), and completion of multi-year multi-locations tests. Agreement with pre-breeders to deliver more target germplasm 7. Provide $F_{1:5}$ to Ethiopia, India, and Iran
2018	1. Survey to define PPs and integration at Stage 2 to 3 2. Re-evaluation of Stage 2 test sites to align with PPs 3. Prioritization of PPs with socio-economists 4. Integration of MAS activities at F_4 for two PPs 5. Better integration of wide crosses into breeding	1. Conduction of an online survey to better define PP needs by NARS partners, definition of a selection index and incorporation of it in Stage 2 to Stage 3 selection 2. GGE model using 2017 data. One site Dropped and two added as small plots 3. 25 F_4 populations selected by MAS to attempt enrichment for drought and heat 4. Double the effort by incorporating at least 50 new F_2 populations derived from wild-relatives each year
2019	1. Re-evaluate F_4 multi-location approach (initial material reached Stage 4) 2. Re-define PPs with partners	1. Done in this document to respond to BPAT request 2. Completed 3. Annually done

	3. Re-evaluation of Stage 2 test sites to align with new PPs 4. Assess value of GS 5. Assess value of MAS-enrichment of F_4 6. Move to a stage gate reporting 7. Align to BPAT review	4. Initial GS germplasm distributed as international nursery Stage 3 5. Activity completed, germplasm to be evaluated in yield trials next season 6. On-going, done for the first time as slide presentation at BCIP steering meeting 7. Completed. Investments are still ongoing
2020	1. Incorporate selection index and GY at TPE in all Stages of advancement 2. De-prioritize PPs that better fit CIMMYT germplasm (high-input environments) 3. Incorporate fast-segregating populations advancement in Mexico 4. Integrate Stage 4 germplasm into 'one CRP WHEAT nursery' 5. Strengthen on-farm data use 6. Validate the use of GS 7. Validate several KASP markers for MAS and increasing Hessian fly resistance in the germplasm 8. Confirm contribution of CWR genome into wide crosses 9. Improve TPE definition with climatic variables	1. Fully incorporated 2. Done for irrigated environment and high-moisture areas 3. Started in 2020, not discussed in this document 4. It is currently done at Stage 5 5. The DIIVA project allowed to incorporate Moroccan farmers' inputs. More is needed for other countries 6. First entries selected by GS included in Stage 3 international nurseries 7. Several KASPs validated including Hessian fly used for F_4 selection 8. Published 9. Ongoing activity with climatologists

Table 2. Brief summary of program growth

Year	National Staff	Area*	Equipment
2013	1 senior technician (Lebanon)	8 ha	Seconded from the French public research institute, INRA, in Morocco
2014	1 senior and 1 junior technician	10 ha	Purchase of 2 combines and 1 planter
2015	1 senior, 3 juniors, 1 pathology consultant	13 ha	Purchase of vertical threshers, seed cleaning unit and automatic seed counter
2016	1 senior, 3 juniors, 1 pathology consultant	14 ha	New seed storage units, purchase of zero-till planter
2017	1 senior, 3 juniors, 1 pathology consultant	15 ha	Establishment of the quality lab
2018	2 senior, 3 juniors	21 ha	Purchase of polytunnel for crosses
2019	2 seniors, 5 juniors, 3 associated breeders, 1	34 ha	Purchase of a semolina mill and pasta makers

	pathology and 1 BMS consultant		

*Calculated as direct breeding activities (yield trials + F generations) only, including salaries

Traits prioritization via product profiles

ICARDA's new strategy document 2018-2026 defined two *mega PPs*: food security (SDG2: zero hunger) and poverty reduction (SDG1: no poverty). These critical profiles were derived from the work of ICARDA's socio-economists who indicated that:

PP based on ICARDA's strategy: food security (SDG2)

Farms with less than 2.0 ha of cropland within the target region of ICARDA serve to sustain an average of four or more adults and six children. Considering average yields below 3 t ha^{-1}, the production is almost entirely consumed in the household and only a small portion is sold to the village market. The use of mixed farming systems incorporating small ruminants is the key to resilience and profitability, and therefore these farmers have a great interest in biomass and taller varieties for straw. These farms have minimum access to credit, so tend to purchase 'good' seeds (rather than 'certified' seeds) of varieties from their neighbors and invest in minimum quantities of fertilizers. Other chemicals are seldom used. Even a 20% raise in their productivity would not be sufficient to harvest sufficient quantities to sell these to the industrial market, but only to the village market. From a breeding standpoint, the key general traits for this mega PP are: tolerance to drought/heat, strong disease resistance packages, large and bold grains to fetch better prices at the local village market, and high biomass to be used as feed.

PP based on ICARDA's strategy: poverty alleviation (SDG1)

Farms with more than 2.0 ha of land have typically good access to seeds and fertilizer, and fungicides are commonly used to protect their fields. Access to supplemental irrigation is also possible. Any excess production is typically sold to grain brokers and industrial markets for transformation into food products. Ensuring a high, industrial quality of grains is therefore directly linked with the ability to increase income and create job opportunities. Hence, the key general traits for this mega PP are*:* tolerance to drought/heat based on the recurring stress of the geography, very strong industrial quality combination (grain size, gluten strength, grain color), and biomass remains also of interest.

Refining PP based on NARS partners' requests

In 2017, an online survey was conducted among NARS partners (Fig 8) to better define what the top priority traits were within each mega PP. After principal component clustering, 15 PPs were identified.

These PPs were discussed with the ICARDA management, aligned to the organizational strategic outcomes (food security and poverty reduction), and weighted by potential rate of impact. A total of eight PPs were prioritized for 2018 and 2019 (Table 3).

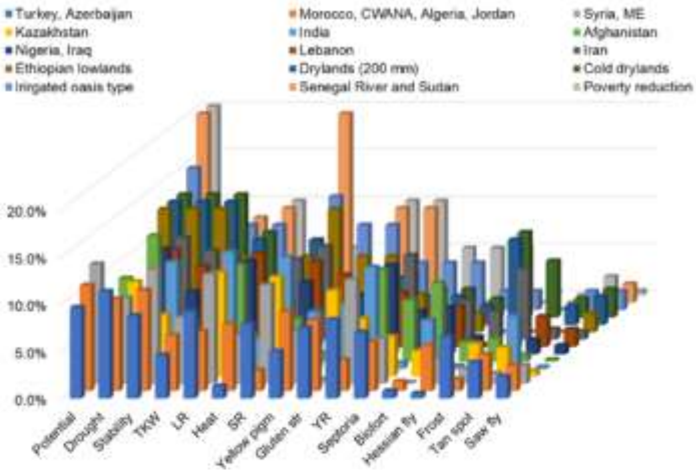


Figure 8. Result of online survey of NARS partners conducted in 2017

Table 3. PPs prioritized in 2018-19 season

PP ID	Type	Country	Main test station	Drought tol	Grain size	Grain color	LR	SR	Heat tol	Gluten str	HF	Frost tol	ST
P1	Food Security	Ethiopian lowlands	Debre Zeit, Sidi el Aydi										
P2	Food Security	Cold drylands (300-200)	Kfardan, Annoceur										
P3	Food Security	Morocco, Algeria, Jordan	Marchouch, Sidi el Aydi										
P4	Poverty Reduction	India	Amalaha										
P5	Poverty Reduction	Senegal River and Sudan	Fanaye, Kaedi, Tessaout										
P6	Poverty Reduction	Drylands medium farms	Marchouch, Allal Tazi										
P7	Poverty Reduction	Lebanon	Terbol										
P8	Poverty Reduction	Iran	Terbol, Allal Tazi										

LR, leaf rust; SR, stem rust; HF, Hessian fly; ST, *Septoria tritici*.
Intensity of grey color determines the level of importance, from low to high.

Strategy for the prioritized PPs for 2018 and 2019

1. Food security in lowlands of Ethiopia: provide partners with $F_{1:5}$ that have undergone two summer cycles under heavy stem rust infection in Lebanon for direct selection under the target environment.

2. Food security in cold Drylands (Atlas Mountains and Iran): use the cold stations (Annoceur and Kfardan) to select under the target conditions. Maintain a small facultative winter-breeding pipeline by selecting germplasm that does not flower in the summer off-season.

3. Food security in Algeria, Jordan and Morocco: this is a core activity of ICARDA. Ensure stress tolerance and disease resistance.

4. Poverty reduction in India: use the Amlaha station to select ideal germplasm for one or two irrigation conditions. Provide also $F_{1:5}$ populations to maximize adaptation.

5. Poverty reduction along the Senegal River: test Stage 3 and Stage 4 material under the Senegal River conditions for direct release. Include also $F_{1:5}$ segregating material for direct selection. Use genomic sequencing to derive the next level of germplasm.

6. Poverty reduction in Drylands of medium farmers: this is a core activity of ICARDA. Use Marchouch station to test Stage 1 and Stage 2 entries across sites. Ensure good industrial quality characteristics.
7. Poverty reduction in supplemental irrigated Lebanese farms: this is a core activity of ICARDA. Use Terbol station to test Stage 1 and Stage 2 entries across sites. Ensure good industrial quality characteristics.
8. Poverty reduction in Iran: test Stage 2 in Terbol, and Stage 3 and Stage 4 material directly in Iran. Include also $F_{1:5}$ segregating material for direct selection. ***This PP was abandoned in January 2019***

Key traits to be selected for at F_1 to Stage 1 to align with the eight PPs

1. Phenology: early and medium
   Visual selection at F_2, F_4, Stage 1.

2. Height: medium and medium-tall (no lodging at above 6 t ha^{-1} yields)
   Visual selection at F_2, F_4, Stage 1.

3. Biotic: Leaf rust and stem rust
   Selection for stem rust at F_1, F_3, F_5 (off-season). For leaf rust apply selection at F_2, F_4, Stage 1.

4. Abiotic: Drought, heat, frost (limited)
   Visual selection for spike filling (grain size) under drought conditions at F_1, F_2, F_3, F_4, F_5, Stage 1. Visual selection for spike fertility (number of grains) under heat conditions at F_1, F_3, F_4, F_5. For frost apply visual selection against frost damage in the spike at F_1 (facultative winter), F_4.

5. Quality: Grain size, grain color
   NIR for grain quality (protein/gluten strength/size/color) at F_4. Stage 1 for NIR and SDS for gluten strength.

6. Grain yield
   Unreplicated augmented design with rows columns spatial correction of F_4 and Stage 1. Discard poorest performing entries.

Important traits to be phenotyped at Stage 2 to align with the eight PPs

1. Yield potential and yield stability
   Use statistical models at Stage 2, 3, 4.

2. Biotic: Leaf rust, *Septoria tritici*, and Hessian fly
   Artificial inoculation and glasshouse testing at Stage 2, 3 and 4. At Stage 3 and 4, the material is also tested in Izmir (rust lab) and Beja (Septoria platform).

3. Abiotic: Drought, heat, frost (limited).

Use statistical models to select against the best check at Stage 2, 3 and 4.

4. Quality: Grain size, grain color, gluten strength
   Complete profile at Stage 2, 3, 4 (>2 environments).

New PPs for 2020-2025

Between January and March 2019, several meetings were held with national partners to identify actual PPs to be targeted together. The result of these discussions is summarized in Table 4. This new list aligns remarkably well with the list of eight prioritized PPs for 2018 and 2019. In addition, the type of traits to be targeted are mostly the same as those identified previously: grain yield stability and potential, grain size, yellow grain color, SDS, the three rusts, Hessian fly, *Septoria tritici*, and winter hardiness.

There are also some additional traits that emerged:

- Crown/root rot tolerance is a trait that was not sufficiently prioritized in the past and for which the connection with Australian research can help. A decision was made to invest and focus on this trait.
- Awnless is a trait that gained interest after witnessing the fact that the Australian cultivar *Saintly* was less prone to bird damage. In addition, the absence of the rough awns should reduce the possibility of wounding the ruminant's mouths when used as feed.
- Super-early is important to ensure fitting within rice rotations. For this, *Ouassara* is a good donor parent.
- Red straw and black awns are appreciated by Algerian farmers and others. It is important to start recording this trait starting from Stage 1 trials. *Margherita* and *Waha* are good donors for both traits.
- Low yellow berry is a trait that is mostly controlled by fertilization rates, but indeed some elites are more sensitive than others. It is important to record this trait in all yield trials. Also, it would be ideal to conduct yield trials using two different nitrogen doses to assess the sensitivity levels.

Key traits to be selected for at F_1 to Stage 1 to align with the 2020 PPs

1. Phenology: super-early, early and medium
   Visual selection at F_2, F_4, record value at Stage 1.

2. Height: medium and medium-tall (no lodging at above 6 t ha^{-1} yields)
   Visual selection at F_2, F_4, record value at Stage 1.

3. Biotic: leaf rust, *Septoria tritici*, and root rot (develop system)
   Selection for stem rust at F_1, F_3, F_5 (off-season). For leaf rust, apply selection at F_2, F_4, and Stage 1. For RR, apply selection at Stage 2 only for now; later shift it to F_4.

4. Abiotic: Drought, heat, frost (limited)
   Selection for spike filling (grain size) under drought conditions at F_1, F_2, F_3, F_4, F_5, and Staqe1. Apply visual selection for spike fertility (number of grains) under heat

conditions at F_1, F_3, F_4, F_5. For frost, apply selection against frost damage in the spike at F_1 (facultative winter)> F_4.

5. Quality: Grain size, grain color, low yellow berry
   Test at $F_{1:4}$ and Stage 1 for grain size, color, gluten strength, and mixograph score.

6. Special characters: Awnless, black awns, red straw color
   Visual selection at F_2 and F_4 for awnless in Saintly crosses. Black awns and red straw visual selection in F_4, and record both traits from Stage 1.

Important traits to be selected for at Stage 2, 3, and 4 to align with the 2020 PPs

7. Yield potential and yield stability
   Use statistical models at Stage 2, 3, 4.

8. Phenology and height: super-early, early and medium-flowering and medium and medium-tall
   Record flowering and height at all trials. Select against lodging at high input Stage 2, and Stage 3 trials.

9. Biotic: leaf rust, stem rust, root rot, and Hessian fly
   Artificial inoculation and glasshouse testing at Stage 2, 3, 4. At Stage 3 and 4 also the material is tested in Izmir (rust lab) and Beja (Septoria platform).

10. Abiotic: Drought, heat, frost (limited)
    Use statistical models to select against the best check at Stage 2, 3, 4.

11. Quality: Grain size, grain color, gluten strength, mixograph score, low yellow berry
    Complete quality profile at Stage 2, 3, 4 (2 environments). At Stage 2 include also a zero N trials to select against yellow berry.

12. Special characters: Awnless, black awns, red straw color
    Record awn type, color and straw color at Stage 2, 3, 4.

Table 4. New PP agreements for 2020-2025

EiB ID	Country	Agro-ecology	Yield competitor cultivar	Phenology	Height	Targeted traits	Special trait	Target surface (ha)	Number of farm household	Average yield t/ha)	Potential surplus	Market opportunities	Gender consideration
100184	Algeria	Drylands (200-300 mm)	Aihn Lehma	Early	80-95 cm	TKW, YP, LR, YR	Red straw, balck awns	800,000	200,000	2.0	160,000	Industrial pasta and couscous	Female cooperatives for frikeh
100072	Algeria	High plateaus (200-300 mm)	Cirta	Early	80-95 cm	TKW, YP, SDS, ST	Red straw, balck awns	900,000	300,000	2.2	198,000	Industrial pasta and couscous	Female cooperatives for frikeh
Submitted by INRA-M	Morocco	Unfavorable zones (200-350 mm)	Karim	Early	80-95 cm	TKW, YP, SDS, HF, YR	Vitrousness, drought	400,000	250,000	1.5	60,000	Local trasformation, industrial premium	Female cooperatives of Khemisset, Safi
Submitted by INRA-M	Morocco	Semi-favourbale (500-400 mm)	Marzak CIMMYT	Early	75-95 cm	TKW, YP, SDS, LR	Large adaptation	500,000	200,000	2.5	125,000	Industrial pasta and couscous	Female cooperatives of Beni Mellal
Submitted by INRA-M	Morocco	Semi-favourbale (500-400 mm)	Marzak CIMMYT	Early	75-95 cm	TKW, YP, SDS, ST	Large adaptation	500,000	200,000	2.5	125,000	Industrial pasta and couscous	Female cooperatives of Beni Mellal
Submitted by INRA-M	Morocco	High drylands (200-400 mm)	Kyperunda	Medium	85-95 cm	TKW, YP	Winter hardiness	100,000	100,000	1	10,000	Local trasformation	Female cooperatives of Bullman
100073	Ethiopia	Mid-highlands (optimal moisture)	Utuba	Early	80-95 cm	TKW, YP, SDS, SR, YR	High tillering	500,000	1,000,000	3	150,000	Industrial pasta	Female aggregated grain collectors
100144	Ethiopia	Lowlands (200-300 mm)	Alemthena	Early	75-95 cm	TKW, YP, SR, YR	Drought tolerance	400,000	800,000	1.5	60,000	Local market and industrial pasta	Female aggregated grain collectors
100112	India	Early planting, 1-2 irrigation, MP	HI1531 (BW)	Med/Early	80-90 cm	TKW, YP, SDS	Low yellow berry	150,000	75,000	3	45,000	Local dalhia and pasta industry	TBD
100284	Senegal	Hot steppe irrigated	Amina, Haby	V. early	75-90 cm	TKW, YP, SDS	Super early, awnless	120,000	240,000	3	36,000	Industrial pasta	Female aggregated grain collectors and seed enterprises
100285	SSA and Yemen (various)	Hot steppe irrigated	various	V. early	75-90 cm	TKW, YP, SDS	Super early, awnless	500,000	240,000	2	100,000	Industrial pasta	Female aggregated grain collectors and seed enterprises
10339	Lebanon	Supplemental irrigation	Miki3 CIMMYT	Medium	75-90 cm	TKW, YP, SR, SDS	Black awns	45,000	28,000	3.5	15,750	Industrial pasta	Refugees females as seed producers
Submitted by INRA-T	Tunisia	Semi-arid	Maali	Early	80-90 cm	TKW, RR	Low yellow berry	300,000	100,000	1.2	36,000	Local market and industrial pasta	Female cooperatives for couscous
Submitted by INRA-T	Tunisia	Semi-arid	Maali	Early	80-90 cm	TKW, YP	Low yellow berry	300,000	100,000	1.2	36,000	Local market and industrial pasta	Female cooperatives for couscous
Submitted by INRA-T	Tunisia	Semi-favorable (400-600 mm)	INRAT100 CIMMYT	Medium	75-95 cm	TKW, ST	Low yellow berry	300,000	100,000	2.5	75,000	Local market and industrial pasta	Female cooperatives for couscous

The CIMMYT logo indicates PP for which ICARDA is not going to invest.

EiB, excellence in breeding; TBD, to be decided; YR, yellow rust; HF, Hessian fly; RR, root rot

Targeted population of environments

The breeding program aims to serve several countries and agro-ecologies. In Table 5, the main targets and relative degrees of importance are summarized. Within each mega-environment (ME) there are several PPs which can be defined, and also within each country and ME there are several distinctions due to market aim or farm size.

Table 5. Country and agro-ecologies targeted by ICARDA

ME	Target Countries	Priority level (1>3)	Durum area (Mha)		Wheat "solution" index	Goal
Semi-arid (250-400 mm)	Algeria	1	2.38	0.80	0.59	*Main goal
	Morocco	1		0.40	0.50	*Main goal
	Tunisia	2		0.30	0.52	*Main goal
	Ethiopia	1		0.20	0.70	*Main goal
	India	2		0.20	0.50	
	Jordan	2		0.02	0.54	
	Palestine	1		0.01	0.81	*Main goal
	Syria	1		0.20	0.71	*Main goal
	Pakistan	3		0.10	0.50	
	Libya	1		0.10	0.87	*Main goal
	Yemen	1		0.05	0.80	*Main goal
	Other SSA	1		-	0.80	*Main goal
Semi-arid cold (250-400 mm, frost)	Iran	2	1.17	0.10	0.42	
	Afghanistan	2		0.10	0.68	
	Turkey	3		0.20	0.38	
	Algeria	1		0.50	0.59	*Main goal
	Morocco	1		0.10	0.50	*Main goal
	Lebanon	2		0.01	0.46	
	Jordan	1		0.01	0.54	*Main goal
	Syria	1		0.10	0.71	*Main goal
	Iraq	2		0.05	0.60	
Sub-humid (400-600 mm) and irrigated	Tunisia	3	3.62	0.30	0.52	CIMMYT focus
	Morocco	3		0.50	0.50	CIMMYT focus
	Algeria	3		0.20	0.59	CIMMYT focus
	India	3		0.90	0.50	CIMMYT focus
	Pakistan	3		0.30	0.50	CIMMYT focus
	Lebanon	2		0.02	0.46	ICARDA focus
	Syria	2		0.30	0.71	ICARDA focus
	Afghanistan	2		0.10	0.68	ICARDA activities
	Iraq	2		0.10	0.60	ICARDA activities
	Turkey	3		0.50	0.38	CIMMYT focus
	Ethiopia	2		0.40	0.80	*Main goal
Irrigated Savannahs	Senegal	1	0.2	0.01	0.80	*Main goal
	Mauritania	1		0.02	0.90	*Main goal
	Egypt	3		0.05	0.52	CIMMYT focus
	Sudan	1		0.05	0.65	*Main goal
	India	3		0.07	0.50	CIMMYT focus
	Other SSA	1		-	0.80	*Main goal

Figure 9 reports prioritization of the different countries based on their average wheat yield gap compared to the global 3 tons per ha average, surface area that each rural individual uses to cultivate wheat, and several socio-economic indexes. Their average represents 'wheat as a solution' score, which identifies Libya, Palestine, the South of Morocco and Syria as the primary countries to be targeted. This score is in alignment with Table 5's definition of ICARDA's priorities. In general, all high-input activities are reserved for CIMMYT germplasm, with few exceptions due to project activities, critical importance, or present results.

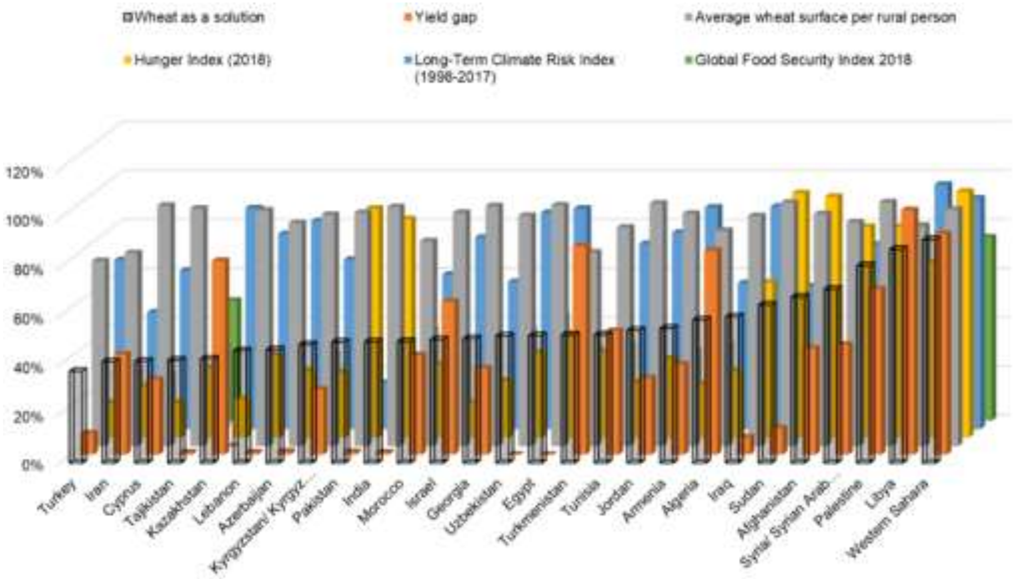


Figure 9. Prioritization of some countries where wheat has the potential to mitigate poverty, hunger and climate change

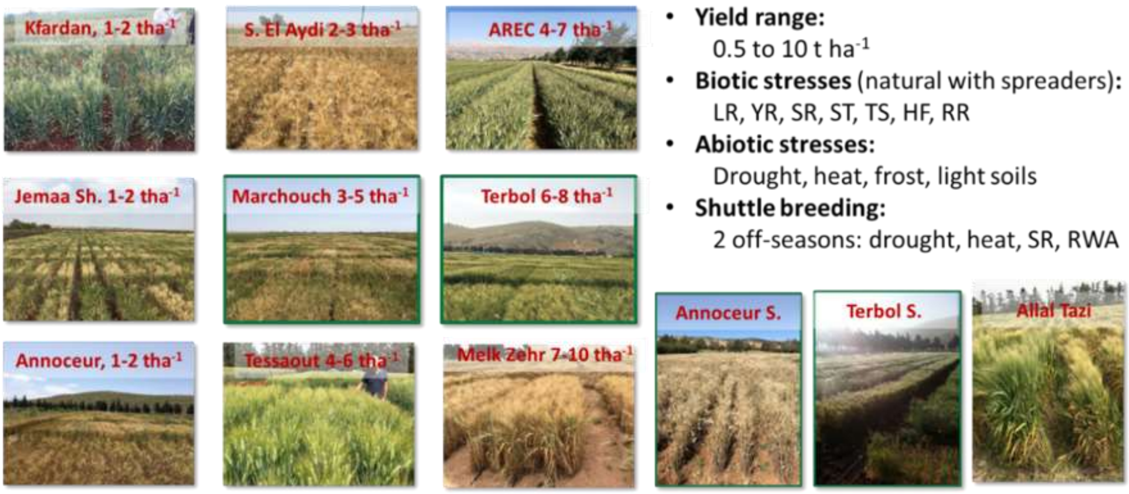
It would not be possible for ICARDA alone to target all these different PPs and agro-ecologies. It is therefore *critical to work with* national breeders in their actual targeted environments at Stage 3 and Stage 4. Representative farm stations need to be used by ICARDA's breeders to select the germplasm until Stage 2 level. To identify these stations, GGE and climatic models need to be applied to define the TPE, a process that allows clustering of the PP target agro-ecologies with representative research stations. This process is best conducted using data from Stage 4 IDYT trials. Research sites that can be easily accessed by ICARDA in Morocco and Lebanon for the F_n, Stage 1 and Stage 2 selections, their characteristics, and generations that have been planted at each site are provided in Table 6. This list of environments was selected to address all the traits that are required in at least one of the PPs. Crown or root rot selection is not yet available and will be developed for Guich via artificial inoculation, starting from 2021.

Stage 4: define the TPE

The Stage 4 IDYT trials represent the ideal test to define the TPE. Table 7 presents the ANOVA (BLUP) for the retrieved information over the past 3 years of Stage 4 testing. In each year, 94 sets of IDYT have been shipped, and feedback was received for 31 to 38 sets. Among these, some partners declared that they could not clear the seeds from custom quarantine in time or presented other reasons for failing the experiment. In many cases, yield data were collected only from a few selected plots, and hence, those data could not be incorporated to run a complete GGE model. In 2016, 2017 and 2018, it was possible to retrieve 19, 25, and 16 sets with complete data, respectively. After statistical analysis, several sites had to be dropped because of poor heritability. The interaction between the different environments is presented in Figure 10.

Table 6. Characteristics of the stations used for breeding selection and summary image

Site	Country	Characteristics
Marchouch	Morocco	Terminal drought, LR, YR, TS, ST
Terbol	Lebanon	Yield potential, YR, SR
Annoceur summer	Morocco	Heat, drought
Terbol summer	Lebanon	Heat, drought, SR
Kfardan	Lebanon	Early/terminal drought, cold, SR
Jemaa Shaim	Morocco	Early/terminal drought, LR, HF
Annoceur	Morocco	Early/terminal drought, cold, TS
Sidi el Aydi	Morocco	Terminal drought, LR, HF
Tessaout	Morocco	Yield potential, heat LR, YR
AREC	Lebanon	Yield potential, SR, LR, YR
Melk Zehr	Morocco	Fertigation, LR, TS, ST
Allal Tazi	Morocco	LR, ST, TS, SR, YR
Guich	Morocco	LR, ST, PM, RR
Izmir	Turkey	YR, SR, LR



LR, leaf rust; YR, yellow rust; TS, tan spot; ST, *Septoria tritici*; SR, stem rust; HF, Hessian fly; PM, powdery mildew; RR, root rot; RWA, Russian wheat aphid.

Table 7. ANOVA for Stage 4 IDYT testing over the last 3 years

GY (kg ha^{-1})	IDYT41 2017-18	IDYT40 2016-17	IDYT39 2015-16
Heritability	0.34	0.62	0.61
Genotype variance	9,826	12,950	27,844
Gen x Loc variance	197,331	90,559	175,896
Residual variance	216,708	215,222	315,745
Grand mean	3,658	2,985	4,151
LSD	228	202	292
CV	12.7	15.5	13.5

n retrieved locations	31	36	38
n locations with yield data	16	25	19
n usable locations	15	14	15

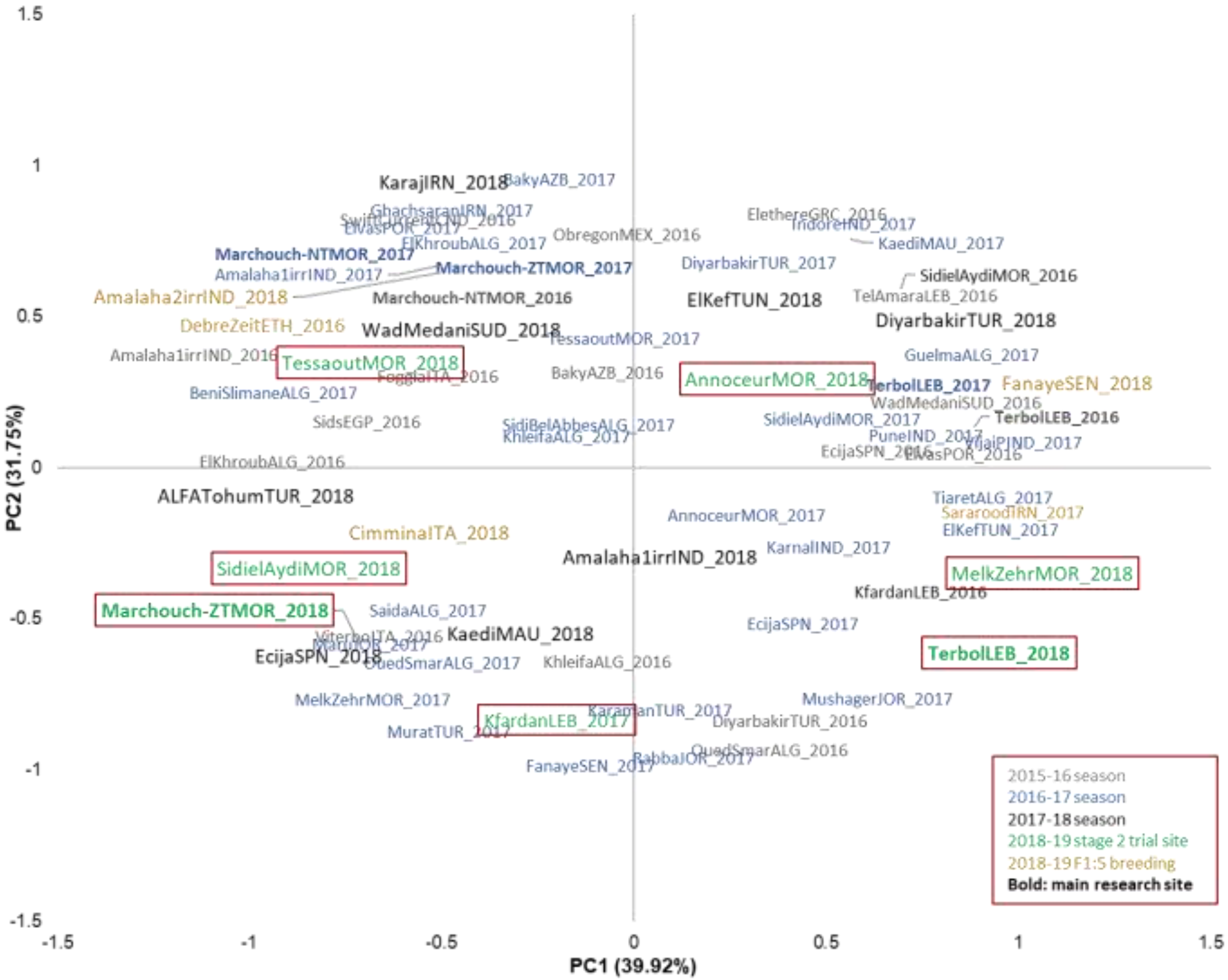


Figure 10. Stage 4 IDYT over 3 years run as GGE of four repeated checks

Table 8. Environment tested over 3 years, heritability, and importance for PP

Site	h2 2018	h2 2017	h2 2016	Type of site	PP link
TerbolLEB	0.59	0.39	0.89	ICARDA site	PP for Middle East
Marchouch-NTMOR	0.64	0.51		ICARDA site	PP for warm Drylands
Marchouch-ZTMOR	0.66	0.77		ICARDA site	PP for warm Drylands
Amalaha1irrIND	0.38	0.38	0.06	ICARDA site, improved	PP for MP area
Amalaha2irrIND	0.29			ICARDA site	PP for MP area
KfardanLEB	0.31	0.69		NARS site: AYT	PP for cold Drylands
AnnoceurMOR	0.33	0.42		NARS site: AYT	PP for cold Drylands
FanayeSEN	0.70	0.54		NARS site: AYT	PP for heat (Senegal/Sudan)
KaediMAU	0.53	0.55		NARS site: AYT	PP for heat (Senegal/Sudan)
IndoreIND	0.35			NARS site: AYT	PP for MP area
SidielAydiMOR	0.37	0.00	0.00	NARS site: improved	PP for Morocco Drylands
TessaoutMOR	0.47	0.00		NARS site: improved	PP for Morocco irrigated lands
ElKefTUN	0.69	0.00		NARS site: improved	PP for Tunisian Drylands
BeniSlimaneALG	0.54			NARS site ok	PP for Algeria
GuelmaALG	0.57			NARS site ok	PP for Algeria
KhleifaALG	0.39	0.48		NARS site ok	PP for Algeria
OuedSmarALG	0.29	0.40		NARS site ok	PP for Algeria
SaidaALG		0.41		NARS site ok	PP for Algeria
TiaretALG	0.16			NARS site ok	PP for Algeria
ElKhroubALG	0.00	0.00		help improve it	PP for Algeria
GhachsaranIRN	0.41			NARS site ok	PP for cold Drylands
KarajIRN	0.70			NARS site ok	PP for cold Drylands
SararoodIRN	0.26			NARS site ok	PP for cold Drylands
DebreZeitETH		0.81		NARS site ok	PP for Ethiopia
WadMedaniSUD	0.75		0.47	NARS site ok	PP for heat (Senegal/Sudan)
MaruJOR		0.86		NARS site ok	PP for Jordanian Drylands
TelAmaraLEB		0.91		NARS site ok	PP for Lebanon
MelkZehrMOR	0.00	0.00		NARS site: Drop	PP for fertigation
SidiBelAbbesALG	0.06			NARS site bad	PP for Algeria
MushagerJOR	0.00			NARS site bad	PP for Jordan
RabbaJOR	0.00			NARS site bad	PP for Jordan
ALFA Tohum TUR	0.79			ICARDA 2nd priority	
BakyAZB		0.00	0.56	ICARDA 2nd priority	
CimminaITA	0.49			Not ICARDA priority	
DiyarbakirTUR	0.81	0.00	0.12	ICARDA 2nd priority	
EcijaSPN	0.65	0.72	0.81	Not ICARDA priority	
ElethereGRC		0.74		Not ICARDA priority	
ElvasPOR		0.77	0.59	Not ICARDA priority	
FoggiaITA		0.92		Not ICARDA priority	
KaramanTUR	0.25			ICARDA 2nd priority	
KarnalIND	0.00			ICARDA 2nd priority	

MuratTUR	0.24			ICARDA 2^{nd} priority
ObregonMEX		0.43		Not ICARDA priority
PuneIND		0.60		ICARDA 2^{nd} priority
SidsEGP			0.59	ICARDA 2^{nd} priority
SwiftCurrentCND		0.83		Not ICARDA priority
VijaiPIND		0.77		ICARDA 2^{nd} priority

Table 8 reports the performances of various sites in terms of heritability and usefulness for the breeding program. For instance, Sidi el Aydi in Morocco was deemed an important station. Its results for 2016 and 2017 showed poor h^2, so an improvement plan for its field management was put in place to significantly raise the quality of the data collected. Using the GGE model among four repeated checks (Omrabi5, Icarasha2, Miki3, Waha) across years, is a possible method by which to analyze Stage 4 yield data and define four major TPE, one for each quadrant of the first two PCs (Fig 9). The sites at which to conduct Stage 2 and F_4 selection can then be defined as those stations with the highest heritability and ease of access within each quadrant.

Using the 2018 data, the following have been selected:
TPE1. Tessaout (MOR)
TPE2. Annoceur (MOR)
TPE3. Terbol (LEB) and Melk Zehr (MOR)
TPE4. Marchouch ZT (MOR), Sidi el Aydi (MOR), Kfardan (LEB).

In addition, five sites were received $F_{1:5}$ germplasm in season 2018-19 to conduct breeding selection directly at the target site. These were:
Independent $F_{1:5}$ breeding 1: Amalaha (IND)
Independent $F_{1:5}$ breeding 2: Fanaye (SEN)
Independent $F_{1:5}$ breeding 3: Sararood (IRN)
Independent $F_{1:5}$ breeding 4: Cimmina (ITA)
Independent $F_{1:5}$ breeding 5: Debre Zeit (ETH).

The following considerations can be made for the main sites looking at the 2018 data:

Marchouch (Morocco)
The introduction of zero-till planting has allowed substantial differentiation of this station with Terbol, and to increase its heritability to above 0.6. The station is a good representative of the North African Drylands.

Terbol (Lebanon)
This is a high-input station with the highest yields. It has heritability above 0.5 and provides a good representation of West Asian sites. The selection conducted here also supports the work conducted in Marchouch.

Kfardan (Lebanon)
This is a drought and cold station, with heritability above 0.4. It typically clusters with Annoceur and away from the two main breeding stations. It represents the high plateaus of Central and West Asia and North Africa (CWANA).

Annoceur (Morocco)
This is a drought and cold station, with heritability above 0.3. It typically clusters with Kfardan and away from the two main stations. It provides a good representation of the high plateaus of CWANA.

Sidi el Aydi (Morocco)
This is a drought station, and an international phenotyping platform, with heritability above 0.3. It typically clusters with Marchouch. It provides a good representation of the North African Drylands.

Tessaout (Morocco)
Tessaout is an irrigated station with high temperatures at the end of the cropping cycle. The management has been improved to reach heritability above 0.4. The station clusters separately from the main stations.

Allal Tazi and Jemaa Shaim (Morocco), and Izmir (Turkey)
These are pest and disease test sites which have been selected for their diversity in pathotypes/biotypes.

Amalaha (India)
One or two irrigations are practiced here. The station has a unique environment and represents its own PP. It does not cluster well with other Stage 2, 3, or 4 sites. A separate $F_{1:5}$ breeding program is run here.

Fanaye (Senegal) and Kaedi (Mauritania)
These are heat-intense irrigated savannahs. They are unique environments and represent their own PP. They do not cluster well with other Stage 2, 3, or 4 sites; a separate $F_{1:5}$ breeding program has been developed for Fanaye and Kaedi.

Debre Zeit (Ethiopia)
This is a disease-intense site. It is a unique environment and represents its own PP. It does not cluster well with other Stage 2, 3, or 4 sites; a separate $F_{1:5}$ breeding program has been developed for it.

A product profile selection index

The 2018 weighted selection index

In order to assign the elite germplasm to different PP pipelines, a weighted selection index was developed to score the matching of Stage 2, 3, and 4 germplasm to the requirements of the national breeders representing different PPs. On the basis of the NARS partners' survey conducted in 2018 (Fig 8), a weighted selection index was developed (Table 9).

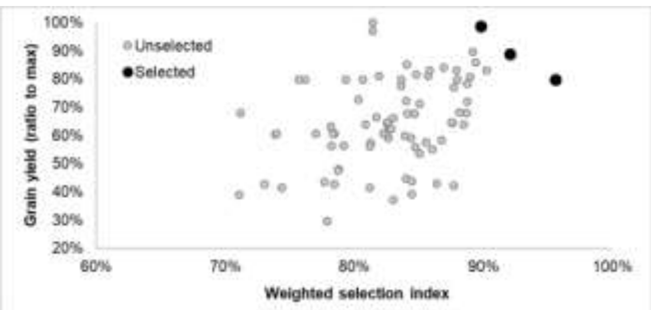
Table 9. PP selection index obtained from the NARS online survey

PP ID	Country	Potential	TKW	Drought	Yellow pigm	Stability	Heat	SR	Gluten str	LR	YR	Frost	Septoria	Hessian fly	Biofort
P1	Ethiopian lowlands	0.18	0.14	0.18	0.05	0.09	0.09	0.14	0.01	0.09	0.05	0.00	0.00	0.00	0.00
P2	Cold drylands (300-200)	0.18	0.14	0.18	0.09	0.09	0.05	0.05	0.01	0.05	0.05	0.14	0.00	0.00	0.00
P3	Morocco, Algeria, Jordan	0.17	0.12	0.17	0.08	0.08	0.08	0.04	0.01	0.08	0.04	0.00	0.04	0.08	0.00
P4	India	0.17	0.13	0.13	0.13	0.09	0.09	0.04	0.09	0.09	0.04	0.00	0.00	0.00	0.00
P5	Senegal River and Sudan	0.20	0.15	0.10	0.15	0.10	0.20	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
P6	Drylands medium farms	0.17	0.13	0.09	0.13	0.09	0.09	0.04	0.09	0.04	0.04	0.00	0.04	0.04	0.00
P7	Lebanon	0.20	0.15	0.05	0.15	0.10	0.05	0.10	0.10	0.05	0.05	0.01	0.00	0.00	0.00
P8	Iran	0.18	0.14	0.09	0.14	0.09	0.05	0.09	0.09	0.05	0.05	0.01	0.05	0.00	0.00
Av		0.18	0.14	0.12	0.11	0.09	0.09	0.06	0.06	0.06	0.04	0.02	0.02	0.02	0.00

A maximum total value of 100% was arbitrary assigned for each of the eight PPs targeted. A total of 14 traits were included, but the weights differentiated the germplasm based on the major four, and minor two to three traits. The remaining traits accounted for less than 5% of the final weight. As such, in the next iteration of selection, only the most critical seven traits will be given a weight.

Efficacy of using a weighted index to select from Stage 3 to Stage 4

In 2017, for the first time, the PP selection index was deliberately used for selecting the elites to be advanced between Stage 3 (international durum observation nursery – IDON) and Stage 4 IDYT trials. Besides the value of the selection index, the yield at the most representative site (Fig 11) was also included to determine the selection, together with the matching of the flowering time.



IDON40	IDYT41	PP 1				
		Ethiopian lowlands				
		Food Security				
		Debre Zeit, Sidi el Aydi				
		IDYT 41	**CB**	**Weig score**	**GY site**	**Flw+Height**
52	Zaghramy	1	1	0.81	0.90	1
95	Swaghost	1	1	0.79	0.80	1
48	Shoukran	1	1	0.79	0.78	1
24	Zouina Bzaff	0	1	0.80	0.99	0
34	Wasihun	0	1	0.83	0.89	0
96	Sissem	0	1	0.86	0.80	0
6	Berghisyr	0	0	0.72	0.97	0
82	Jigéenji Dex (L	0	0	0.76	0.68	0

Figure 11. Combining selection index, grain yield at the target site, and phenology

The black circle represents the Stage 3 elite selected to become part of the Stage 4 trial.

The use of the weighted selection index and the grain yield value recorded at the site most representative of the TPE allows the identification of Stage 3 entries that better fit the PP. However, only those entries that also match the right phenology are advanced to Stage 4. Instead, those that have top values, but the wrong phenology, are recycled into the crossing block (CB).

Comparison of the value obtained for the same index applied at Stage 3 versus the score obtained in Stage 4 for each of the eight targeted PP is presented in Figure 12. The accuracies between the two test stages are extremely low. However, when looking at the top three entries for each PP, it was possible in all cases to identify at least one entry in common between the two test stages.

In the case of PP1, PP3, PP4, PP5, PP6, PP7, and PP8, the absolute top one entry was the same between the two test stages. Only for PP2 was this not the case, but it could be argued that this is also one of the PPs for which less emphasis was placed at the Stage 3 selection. It can be concluded that the selection index was indeed capable of enriching Stage 4 for elites ideally suited to represent the targeted PP, even though some efforts need to be incorporated to increase the accuracy of the selection from Stage 3 to Stage 4.

Another measurement of the accuracy of the process of selection from Stage 3 to Stage 4 is to determine how many of the entries defined as belonging to a specific PP could be confirmed to match that specific PP when selected from the Stage 4 to Stage 5 (National Trial) after testing on the actual target environment (Table 9). With the exception of PP2 (food security for the cold Drylands), for all other PPs, at least one entry could be confirmed. For PP3 to PP8, it was possible to confirm more than one with a matching ratio above 50%. For PP1 (food security in lowlands of Ethiopia) only one entry (33% matching) could be confirmed. Still, since 2017, Ethiopian breeders have received $F_{1:5}$ populations, which allows direct selection to be conducted within the target environment to increase the chances of finding best-fit germplasm.

However, not all of the eight PPs selected for in 2017-18 and 2018-19 are considered with equal importance. Therefore, different numbers of entries were selected for each. In particular, PP4 (poverty reduction in India), for which ICARDA partners with the Indian Agricultural Research Institute and the government of Madya Pradesh, and PP7 (poverty reduction in supplemental irrigated Lebanese farms), which has been recently threatened by the appearance of stem rust disease, were deemed of greater importance and, for these, seven Stage 3 elites were selected (Fig 13). Similarly, PP1 (food security in lowlands of Ethiopia) and PP5 (poverty reduction along the Senegal River) have also been judged of great importance, and six elites each were selected to match these PPs. Along the same lines, PP3 (food security in Algeria, Jordan and Morocco), PP6 (poverty reduction in the Drylands of medium farmers), and PP2 (food security in cold Drylands) are core activities of ICARDA and hence five, four, and three Stage 3 elites were selected to be integrated within the Stage 4 trials, respectively. Finally, PP8 (poverty reduction in Iran) was supported by a project targeting that country, which unfortunately was closed in 2019. As such, only two

Stage 3 entries were included, and this PP will be abandoned from 2019 onward. Only one entry (Shabrach Red) was not selected on the basis of the PP-weighted index. This special elite has red grains and is top quality for the production of burghul wheat. As such, it is of great interest for Turkey and other Asian countries; while these regions are less targeted by ICARDA, it was deemed useful to provide one targeted entry as part of the Stage 4 elites. Except for this last entry, a total of 15 x Stage 3 elites (IDON) were selected to match the eight prioritized PPs. This result provides several indications for the future deployment of elites in the breeding program:

- The breeding pipeline allows selection for all main targeted PPs, and even allows for the selection of lines that match more than one.
- As expected, the crossing scheme and overall profile pipeline are not yet well developed within ICARDA and therefore there is still low specialization of elites to match only one PP.
- The Stage 3 to 4 accuracy is very low. More complex GxE models should be used.
- For those PPs for which it is possible to mimic the target environment, it would be good to incorporate a selection step at the representative environment in F_4, and Stage 1 also.
- For those PPs for which it is not possible to mimic the target environment, it would be good to develop a specialized $F_{1:5}$ pipeline:
- PP2: a facultative winter program was developed to specifically target it, and the resulting first set of F_6 will be assessed next season
- PP1 (lowlands of Ethiopia)
- PP5 (Senegal River)
- PP4 (Madya Pradesh)
- PP8 (Iran)

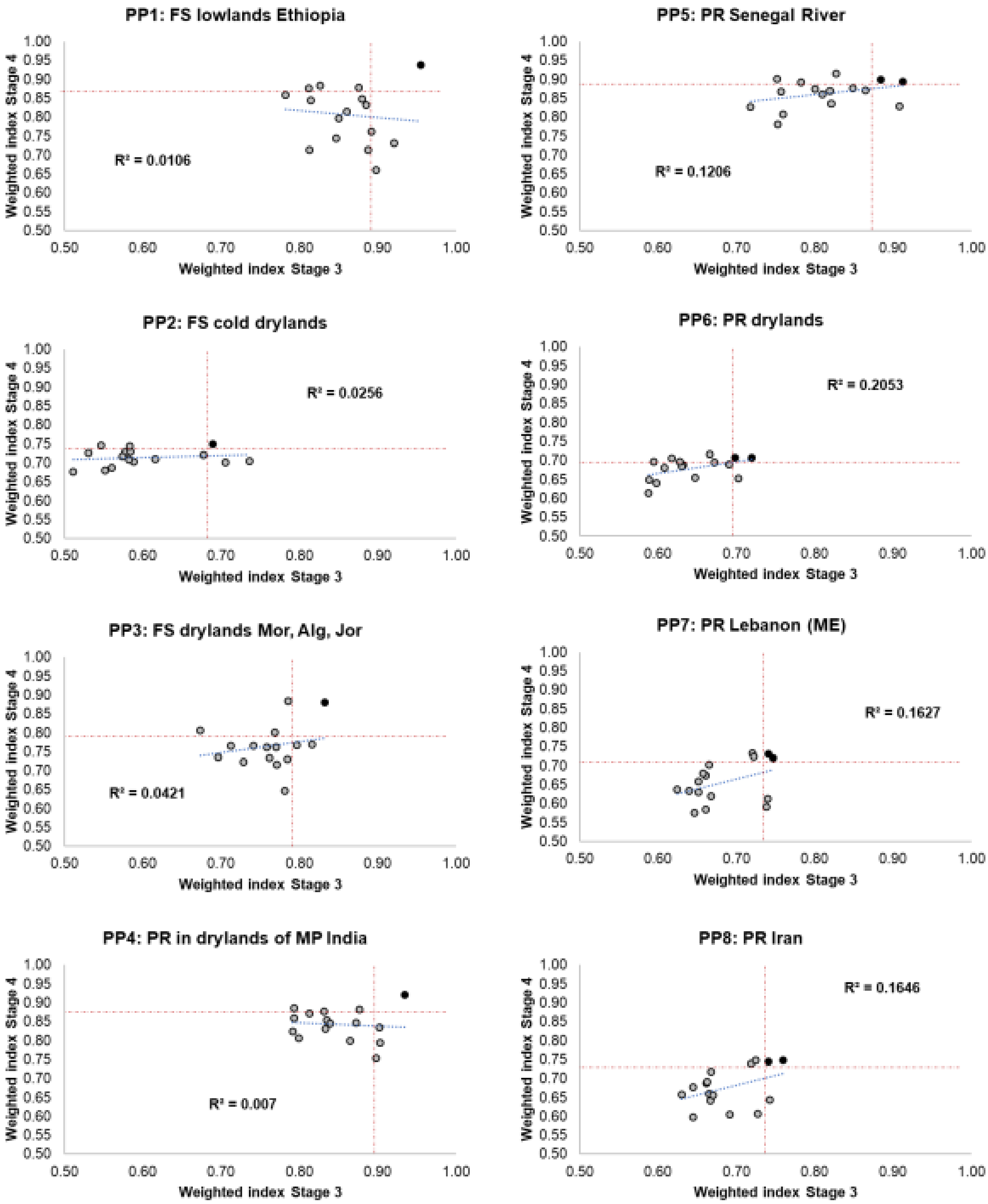


Figure 12. Comparison of weighted index values between Stage 3 and Stage 4 phenotyping for each of the eight main PPs

The prediction accuracies (r^2) are reported on the graph. The red dashed lines define the top three entries for Stage 3 (vertical) and the top three entries for Stage 4 (horizontal). Entries that were among the top three for both Stages have been color-coded as black dots.

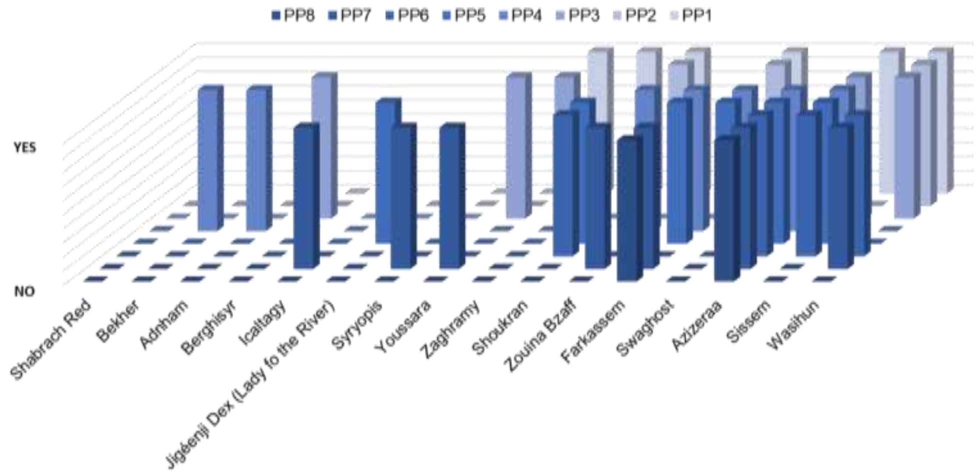


Figure 13. Stage 3 IDON selected entries to be tested in Stage 4 IDYT and their matching to each targeted PP

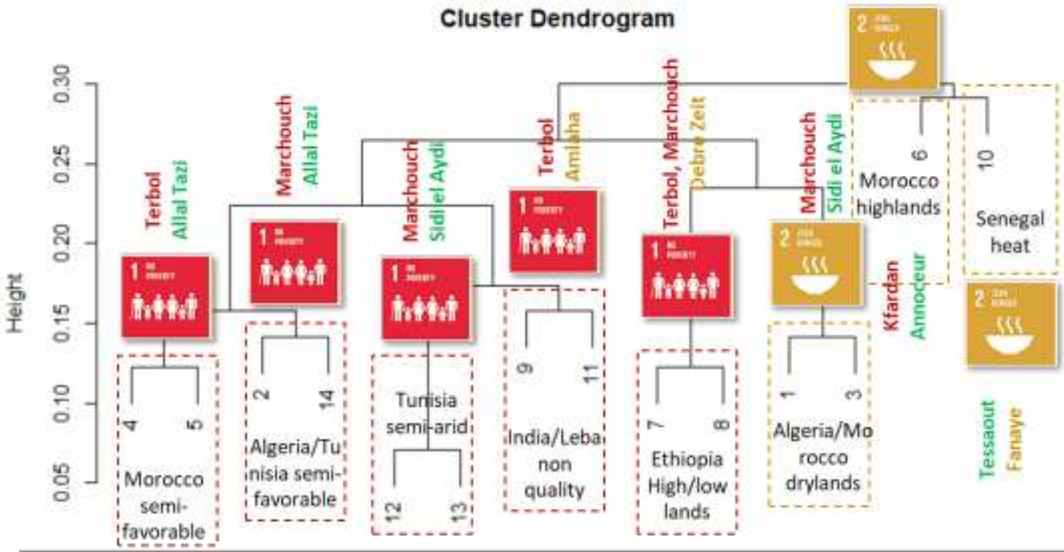
A value of 1 means matching the specific PP above 85% weighted index.

The 2020 weighted selection index

A total of 14 PPs associated with 15 traits were identified in the NARS survey conducted in 2019 (Table 4) and a focus on these PPs began in the selection season 2019-2020. A weighted selection index was developed for each PP (Table 10) assigning 20% weight to yield potential, 15% to the secondary trait, 10% to the tertiary trait, 5% to minor traits, and 0% to traits of no/low interest. To understand how many true PPs are represented by these 14 requests, a principal component analysis was run using the selection index as a discriminatory value, to reveal eight unique mega profiles, five linked to poverty reduction and three to food security (Fig 14).

Table 10. Selection index for the 2020 PPs

	Country	Agro-ecology	Yield potential	TKW	Drought	Yellow pigment	Stability	Heat	SR	Gluten str	LR	YR	Frost	Septoria	Hessian fly	Root rot	yellow berry	Flowering	Height
P1	Algeria	Drylands (200-300 mm)	0.20	0.15	0.10	0.10	0.15	0.00	0.00	0.00	0.10	0.10	0.00	0.00	0.05	0.05	0.00	Early	80-95 cm
P2	Algeria	High plateaus (200-300 mm)	0.20	0.15	0.10	0.10	0.15	0.00	0.00	0.10	0.05	0.00	0.00	0.10	0.00	0.00	0.05	Early	80-95 cm
P3	Morocco	Unfavorable zones (200-350 mm)	0.20	0.15	0.10	0.10	0.10	0.00	0.00	0.10	0.05	0.10	0.00	0.00	0.10	0.00	0.00	Early	80-95 cm
P4	Morocco	Semi-favourbale (500-400 mm)	0.20	0.15	0.00	0.10	0.15	0.00	0.05	0.10	0.10	0.05	0.00	0.05	0.00	0.00	0.05	Early	75-95 cm
P5	Morocco	Semi-favourbale (500-400 mm)	0.20	0.10	0.00	0.10	0.10	0.00	0.05	0.10	0.10	0.05	0.00	0.15	0.00	0.00	0.05	Early	75-95 cm
P6	Morocco	High drylands (200-400 mm)	0.20	0.15	0.10	0.15	0.15	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.10	Medium	85-95 cm
P7	Ethiopia	Mid-highlands (optimal moisture)	0.20	0.15	0.00	0.10	0.15	0.00	0.15	0.10	0.00	0.10	0.00	0.00	0.00	0.05	0.00	Early	80-95 cm
P8	Ethiopia	Lowlands (200-300 mm)	0.20	0.15	0.10	0.10	0.15	0.00	0.10	0.05	0.00	0.10	0.00	0.00	0.00	0.05	0.00	Early	75-95 cm
P9	India	Early planting, 1-2 irrigation, MP	0.20	0.15	0.10	0.10	0.10	0.00	0.10	0.10	0.05	0.00	0.00	0.00	0.00	0.00	0.10	Med/Early	80-90 cm
P10	Senegal	Hot steppe irrigated	0.20	0.15	0.00	0.15	0.15	0.20	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.05	V. early	75-90 cm
P11	Lebanon	Supplemental irrigation	0.20	0.15	0.00	0.15	0.15	0.00	0.10	0.15	0.00	0.00	0.00	0.00	0.00	0.05	0.05	Medium	75-90 cm
P12	Tunisia	Semi-arid	0.20	0.15	0.05	0.10	0.15	0.00	0.00	0.10	0.05	0.00	0.00	0.00	0.00	0.10	0.10	Early	80-90 cm
P13	Tunisia	Semi-arid	0.20	0.15	0.10	0.10	0.15	0.00	0.00	0.10	0.05	0.00	0.00	0.00	0.00	0.05	0.10	Early	80-90 cm
P14	Tunisia	Semi-favorable (400-600 mm)	0.20	0.15	0.00	0.10	0.15	0.00	0.05	0.05	0.05	0.00	0.00	0.15	0.00	0.00	0.10	Medium	75-95 cm



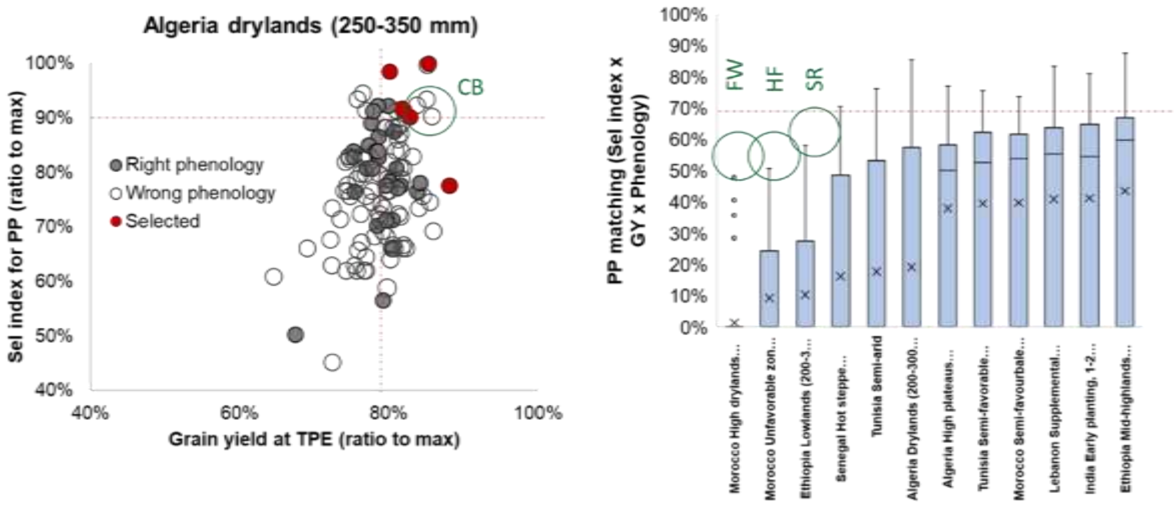
	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14
P1	0.68	0.84	0.71	0.49	0.60	0.60	0.80	0.59	0.43	0.51	0.68	0.72	0.51
P2		0.71	0.84	0.79	0.67	0.56	0.68	0.80	0.60	0.72	0.80	0.88	0.85
P3			0.70	0.51	0.51	0.62	0.75	0.66	0.49	0.60	0.63	0.71	0.45
P4				0.87	0.55	0.78	0.71	0.79	0.62	0.83	0.80	0.80	0.84
P5					0.35	0.57	0.49	0.60	0.44	0.63	0.57	0.57	0.88
P6						0.43	0.60	0.66	0.52	0.57	0.67	0.73	0.57
P7							0.89	0.70	0.52	0.87	0.64	0.60	0.58
P8								0.75	0.50	0.76	0.68	0.72	0.54
P9									0.55	0.83	0.80	0.88	0.69
P10										0.68	0.63	0.63	0.54
P11											0.83	0.79	0.69
P12												0.96	0.70
P13													0.70

Figure 14. Correlation matrix between the 2020 PPs based on the weighted selection index

For each PP, the figure above reports the type (food security or poverty reduction) and the station to be used for Stage 1 and Stage 2 testing. The main ICARDA stations are in red, the Stage 2 sites in green, and the sites where independent $F_{1:5}$ selection is conducted are highlighted in yellow.

On the basis of the good results obtained from the application of the selection index and GY at the TPE, this methodology was deployed for the selection of Stage 2 to Stage 3, and from Stage 3 to Stage 4 (Fig 15). This strategy allowed for rapid identification of the best-matching entries for each PP, and also for a definition of those entries that had all desired traits but failed to match the required phenology. Those that failed were then destined for the CB.

Stage 2 to Stage 3 2019



Stage 3 to Stage 4 2019

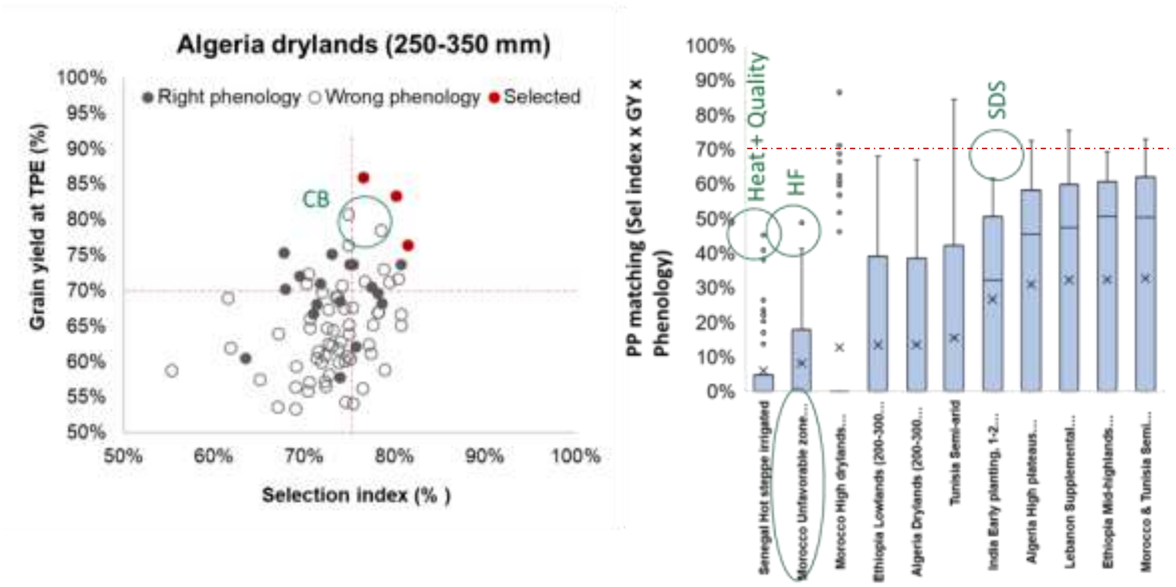


Figure 15. Results for the Algerian Drylands PP for combining the selection index (horizontal axis) and GY at the TPE (vertical axis)

Comparison of Stage 2 to 3 and Stage 3 to 4 advancement in season 2018-19, together with the number of entries matching each mega PP. The entries with the wrong phenology are presented as empty circles, the selected entries are color coded in red. The traits that were lacking from the different stage entries are shown in green in the figure to the right.

For the first time in 2018-19, a PP matching score was deployed, which was obtained by multiplying the percentage values of GY at the TPE, selection index, and right phenology (Fig 15 right). A score of 70% is considered as a good match for each PP. This allowed confirmation that for most of the PPs, it was possible to identify several entries that matched the requirements. On the other hand, it was also possible to define those PPs for which the current breeding method could not deliver ideal elites, and for which it is then necessary to define alternative strategies.

In the case of the Stage 2 entries tested in 2018-19 season, the PP for the Moroccan high-Drylands could not be properly served due to a lack of ideally-matching phenology (facultative winter). In that sense, the deployment of a parallel breeding effort aimed at facultative winter is fully justified. Surprisingly, the Moroccan unfavorable PP, which represents a historical target of ICARDA's breeding effort was also poorly served both in Stage 2 and Stage 3 tests. This was due to a low number of entries having good resistance to the Hessian fly, as well as top industrial quality and other desirable traits. For that reason, a molecular enrichment strategy utilizing the recently validated QHara.icd.6B KASP marker needs to be deployed.

Ethiopian lowlands are also a main target, and in this case, the main issue was the low frequency of stem rust-resistant entries that were also drought tolerant and of high quality. In that sense, the deployment of a parallel breeding effort in Ethiopia using $F_{1:5}$ segregating populations appears fully justified. It is also important to note that in Stage 3 tests, there were two extra PP that were poorly served. There were Senegal River Hot Steppes due to the low number of entries combining good industrial quality with heat tolerance; and for P9 (India early planting one-two irrigations) due to the low number of entries with good water use efficiency and strong gluten. Both these issues were resolved in Stage 2 trials, confirming that the deployed breeding strategy worked well in this case.

Stage-to-gate to deliver PP traits via breeding

Figure 16 illustrates the overall strategy for deploying a PP breeding strategy and the basic steps involved in the Stage-to-gate breeding pipeline, where 'stage' relates to the steps involved, and 'gate' to the farmers' fields. The first step is to define clear 35 year goals in terms of PPs with the national breeders. These goals are then grouped into mega PPs as shown in Figure 14 and their priority is set based on a multi-disciplinary team as described in Table 5 and Figure 9**.** The PPs then need to be validated by the stakeholders that will be involved in their use (farmers, industry, and others) via meetings, surveys, and value-chain analysis. At this point, the actual breeding work starts with the definition of the TPE (Fig 10) and of the PP-related selection index (Table 9 and Table 10). Finally, a breeding model is defined as 'Stage-to-gate' to reach commercialization as fast as possible, whilst keeping costs to a minimum. The 'Stages' involved are reported in Figure 16 (right) and the details are presented in the following paragraphs.

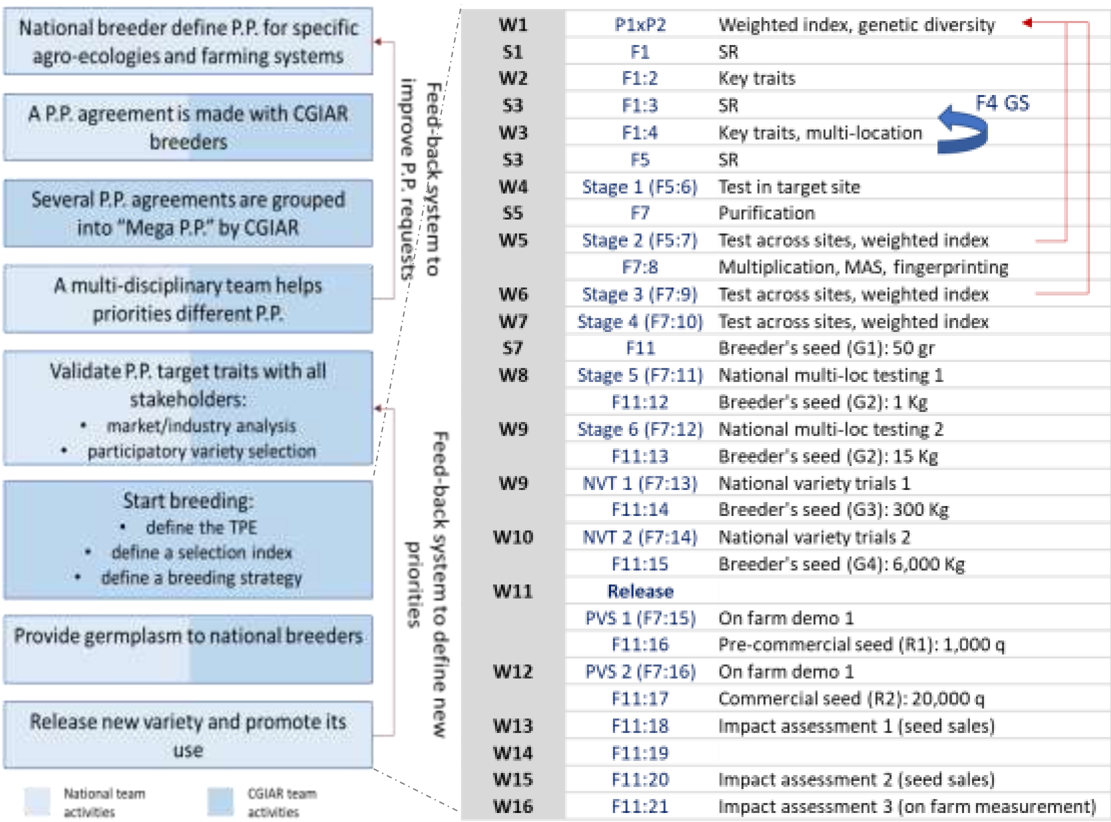


Figure 16. Overall PP strategy and the Stage-to-gate breeding method used by ICARDA for the release and commercialization of varieties

Briefly, the Stage-to-gate scheme is based on several key stages. The PP matching score is used to define ideal mating elites selected from the Stage 2-3-4 entries. The advancement to F_5 is accelerated by using shuttle breeding, recombinant GS, and then by the deployment of speed-breeding targeting only the key traits common to all PPs (see Key traits to be selected for at F_1 to Stage 1 section). The Stages 2-3-4 are advanced on the basis of PP matching scores. The following Stage 5 and Stage 6 trials are conducted at the TPE by national breeders following the list of prioritized traits in the signed PP agreements. While the germplasm undergoes national catalogue registration, the seeds are purified to achieve 6 tons of G_4 breeders' seed by the time the variety is registered. Then, while the commercial seed is produced, the national breeders engage in several participatory variety selection demonstrations to stimulate farmers' interest. Ultimately, the impact of the breeding activities is assessed in terms of the market gained, and the participatory activities are used to identify any remaining gaps between what was proposed and what is required by farmers. These gaps are then incorporated into new PP agreements and the process starts anew.

Comparison of different breeding methodologies

Three main breeding methodologies are well suited for spring durum wheat: single seed descent by speed breeding; single seed descent using a glasshouse; or selected bulks using off-season shuttle breeding. The use of double haploids does not provide a significant advantage in spring wheat. Table 11 summarizes some of the costs and steps involved to go from crossing to the first yield trial using the three methodologies.

Table 11. Comparison of breeding methodologies

	Speed breeding			Single seed descent			Selected bulks		
Season	Generation	Investment	Stage	Generation	Investment	Stage	Generation	Investment	Stage
W1	P x P	Field	Cross	P x P	Field	Cross	P x P	Field	Cross
S1.1	F1	Fast GH	SSD	F1	GH	SSD	F1	Off-season	Bulk
S1.2	F1:2	Fast GH	SSD						
W2.1	F1:3	Fast GH	SSD	F1:2	GH	SSD	F1:2	Field	Sel bulk
W2.2	F1:4	Fast GH	SSD	F1:3	GH	SSD			
S2	F5	Off-season	Multipl	F1:4	GH	SSD	F1:3	Off-season	Bulk
W3	F5:6	Field	1st yield trial	F5	Field	Multipl	F1:4	Field	Mult-loc
S3							F5	Off-season	Multipl
W4				F5:6	Field	1st yield trial	F5:6	Field	1st yield trial

Investment	Unit x fam	Unit cost	Use	Cost	Use	Cost	Use	Cost
Field	9m2 plot	10	2	20	2	20	3	30
Off-season	2 m2 plot	5	1	5	0	0	2	10
GH	0.2 m2 bench	15	0	0	4	60	0	0
Fast GH	0.2 m2 bench	20	4	80	0	0	0	0
Total				**105**		**80**		**40**

Selected bulk using off-season:

This method relies on the use of field selection at F_2 as bulked spikes, and pedigree in F_4 of 12 individual F_5 plants per population (instead of 48). It uses an off-season site to advance during the summer, and provides the advantage that all steps occur under field selection and that F_4 multi-locations testing can be conducted. A surface of 10 m^2 is required per population in winter and the current overhead for land and soil during this time is US$10 per plot or US$10,000 per ha. For 2 m^2 during the off-season, the cost is US$25,000 per ha. Based on these assumptions, this approach would reach the first yield trial in 4 years, and cost US$ 40 per population, excluding marker and pathology selections.

Single seed descent:

The single seed descent (SSD) methodology relies on the use of normal glasshouse or plastic houses, with the ability of completing three seasons per year. One population advanced by SSD using this method requires a minimum population size of 48 individuals as no phenology selection can be conducted prior to being grown out in the field. On the other

hand, this method is well suited for the imposition of selection with molecular markers and artificial pests' inoculations. A bench space of 0.2 m^2 is required per population, or 20 m^2 for 100 populations. The current overhead for salary, soil, and electricity is US$75 per m^2. Further, field trials and field staff need to be maintained to ensure the actual yield testing and crossing step. One additional comment is that 48 individuals per population need to be planted and selected for as spike-to-row.
Based on these assumptions, this approach would not reduce the time to yield trial, since one step of spike-to-row multiplication in the field would still be required, and it will cost US$80 per population, excluding marker and pathology selections. It might, however, provide an advantage of greater selection intensity with more progenies evaluated per cross at the F_5, while causing a reduction in the number of crosses that can be advanced per year.

Speed breeding by SSD:
This method relies on the use of 24 hours photoperiod and low temperatures to achieve up to six generations per year. However, advancement of six generations in 1 year is not practically achievable at a large scale since, every 2 months, all spikes need to be harvested, moved to an incubator, germinated and transplanted. When this is applied on only a few populations, it is possible, but the turnaround time between steps on a large scale prevents its success. Instead, four generations per year is a reasonable achievement and can be done with a step that includes MAS, GS, and phenotyping for highly heritable traits, such as diseases. One population advanced by SSD under speed breeding requires a minimum population size of 48 individuals, and the imposition of selection pressure with markers and pests. A bench space of 0.2 m^2 is required per population, or 20 m^2 for 100 populations. The current salary, soil, and electricity overheads for a glasshouse with speed breeding capability is US$100 per m^2. In addition, field trials and field staff need to be maintained to ensure the actual yield testing and crossing step. One additional comment is that 48 individuals per population need to be planted and selected for as spike-to-row for multiplication.
Based on these assumptions, this approach would reduce the time to achieve the first yield trial by 1 year, but would cost US$105 per population, excluding marker and pathology selections. However, it might provide an advantage of greater selection intensity with more progenies evaluated per cross at F_5, while causing a reduction in the number of crosses that can be advanced per year.

Following these considerations, the program has decided to continue the use of the selected bulk method with integration of off-season advancement since it allows larger population sizes to be advanced in the field; requires 4 years to reach the first yield trial instead of 3 years by speed breeding; and costs US$40 per population compared to US$80 and US$105 for SSD and speed breeding, respectively.

However, the SSD and speed breeding methodologies are better suited for MAS and GS approaches. As such, these have been integrated for 20 populations per year, targeted to these strategies. In addition, Excellence in Breeding has recently raised funds for CIMMYT to build speed breeding facilities. Consequently, the ICARDA program has agreed to shift all crosses with F_4 advancements to Mexico to accelerate the turnaround time by 1 year, while all steps from F_4 onward remain unchanged. The schematics for running an SSD speed breeding method incorporating GS is presented in the dedicated section.

Deploying the Stage-to-Gate strategy

Standard pedigree and selection history codes

Pedigree codes:

- A/B//C/3/D, where the slash '/' indicates the temporal order from '/' first cross made to '/3/' last one, and the parents presented to the most left is always the one used as female (emasculated) in the hybridization process.
- A*3/B where the star indicates a backcross, the line A is the recurrent parent, and 3 is the number of times the backcross was repeated.
- Lines that reach Stage 4 receive a cross name, and this replaces the full pedigree.

Cross codes:

The standard selection history of a cross should be:
ICD*xNN*-0001

- ICD: cross code stands for ICARDA Durum, which is applied to all crosses made by ICARDA researchers.
- x: indicates the country where the cross was realized, and it can be M, L, I, S, W for Morocco, Lebanon, India, Senegal, or for winter program, respectively. TR was used until 2015 to indicate the work done in Lebanon, but it has been since replaced by L.
- NN: is a two-number code to indicate the year when the cross was made. For instance, '17' would indicate 2017
- -0001: is a value that identifies the specific cross and it varies from 0001 to 1000.

Additional cross codes:

- ICDL18(F2)-0208: the addition of '(F2)' indicates that the cross is in fact a top cross made in F_2. Therefore, the two parents that constitute the male (F_2 is always the pollen donor) contribute only 25% each to the final genome, while the female contributes 50%.
- GRS: this code recognizes the crosses made by the genebank, followed by their own coding system.
- ICDM-GS14-0084: this is an example of a cross made based on the GS value.
- QAF0442: this material was developed by the team of Lee Hickey and Samir Alahamad at the Queensland Alliance for Agriculture and Food Innovation in Australia and advanced to F_5 via speed breeding.
- Crop Trust-NU-2018-BC3F1-1: this material was developed by the team of Ian King at the University of Nottingham by bread wheat Ph1 mutants crossed with wild relatives and then backcrossed to durum wheat elites.
- ICDJMC04-001-BThL (Bulksel): this is an old code to identify wide crosses made by the genebank. It has now been replaced by GRS.
- ICD06-87-BLMSD: this is an old code to identify specific crosses made in Aleppo and it has now been replaced.

- ICDGSM17r1-11: in this case, the cross was made based on GS, and this is the recurrent cycle 1 (r1) line between two full-sibs. As such, it has the same pedigree of the two siblings, but it is in fact a new cross.

Selection history codes:
The standard selection history of a cross should be:
ICD11-129-0TR-7STR-0TR-1TR-0STR-5TR-0STR-0AUB-0AUB

- Starting with the standard cross code, followed by each step of selection until bulking separated by a '-' without any spaces. A number indicates the advancement strategy and two-three letters indicate the field station where the selection occurred.

Selection advancement codes:

- '0': indicates bulking of seeds without selection, such as when harvesting a whole plot.
- '015': indicates a selected bulk, with 15 spikes selected and threshed together. The number might vary i.e. 05, 020, etc.
- '1': indicates a pedigree step where an individual plant or spike was harvested and threshed. The number indicates the "actual" plant selected so: 1 for the first plant, 2 for the second, 3 for the third, etc.

Notice that a bulk advancement (selected or not) means that full heterogeneity is retained, and the following generation is 'derived'. Advancing by bulk reduces heterozygosity of the individual plant but maintains full heterogeneity. A pedigree advancement, on the other hand, means that all population heterogeneity disappears since a single plant is harvested, and a new 'underived' generation is obtained. Table 12 shows the levels of heterozygosity and heterogeneity of the typical pipeline of ICARDA durum.

Table 12. Heterozygosity and heterogeneity of different generations based on bulk or pedigree selection

Gen.	Heteroz.	Heterog.	Gen.	Heteroz.	Heterog.
F1	100.0%	100.0%	**F5:6**	3.1%	6.3%
bulk			pedigree		
F1:2	50.0%	100.0%	**F7 (pre-IN)**	1.6%	1.6%
sel. bulk			bulk		
F1:3	25.0%	100.0%	**F7:8 (Stage 3)**	0.8%	1.6%
bulk			bulk		
F1:4	12.5%	100.0%	**F7:9 (Stage 4)**	0.4%	1.6%
pedigree			pedigree		
F5	6.3%	6.3%	**F10 (G0)**	0.2%	0.2%
bulk			bulk		
F5:6 (Stage1)	3.1%	6.3%	**F10:11 (G1)**	0.1%	0.2%
bulk			bulk		

F5:7 (Stage 2)	1.6%	6.3%	**F10:12 (G2)**	0.0%	0.2%

Selection advancement sites:

- Different sites are used for advancement. Their codes are summarized in Table 13.
- For instance:
  ICD11-129-0STR-0TR-7STR-0MCH[MtAJDSZ]-0AUB: indicates bulking of F_1 in the summer season of Terbol, bulking of F_2 in winter season in Terbol, harvesting by pedigree of the F_3 plant number seven in summer Terbol, followed by bulking in Marchouch winter, and again in American University of Beirut. The final plant would therefore be a $F_{4:7}$, since pedigree occurred from an F_3 pant and therefore F_4 seeds, and then continued by bulking.
- 0MCH[MtAJDSZ]: this is an old code, it meant that the bulk occurred in Marchouch, but that the selection was done at several additional sites. This code has been discontinued.

Table 13. Stations codes used for selection

Code	Station	Country	Code	Station	Country
AUB	American University of Beirut	Lebanon	**ALT**	Allal Tazi	Morocco
KFD	Kfardan	Lebanon	**AN**	Annoceur	Morocco
STR	Summer Terbol	Lebanon	**SAN**	Summer Annoceur	Morocco
TR	Terbol	Lebanon	**GCH**	Guich	Morocco
DBZ	Debre Zeit	Ethiopia	**JS**	Jemaa Shaim	Morocco
AML	Amlaha	India	**MCH**	Marchouch	Morocco
KAD	Kaedi	Mauritania	**MKZ**	Melk Zehr	Morocco
FAN	Fanaye	Senegal	**SA**	Sidi el Aydi	Morocco
IZM	Izmir	Turkey	**TES**	Tessaout	Morocco

A schematic representation of the ICARDA durum wheat breeding program is presented in Figure 17, and is described in detail in the following chapters.

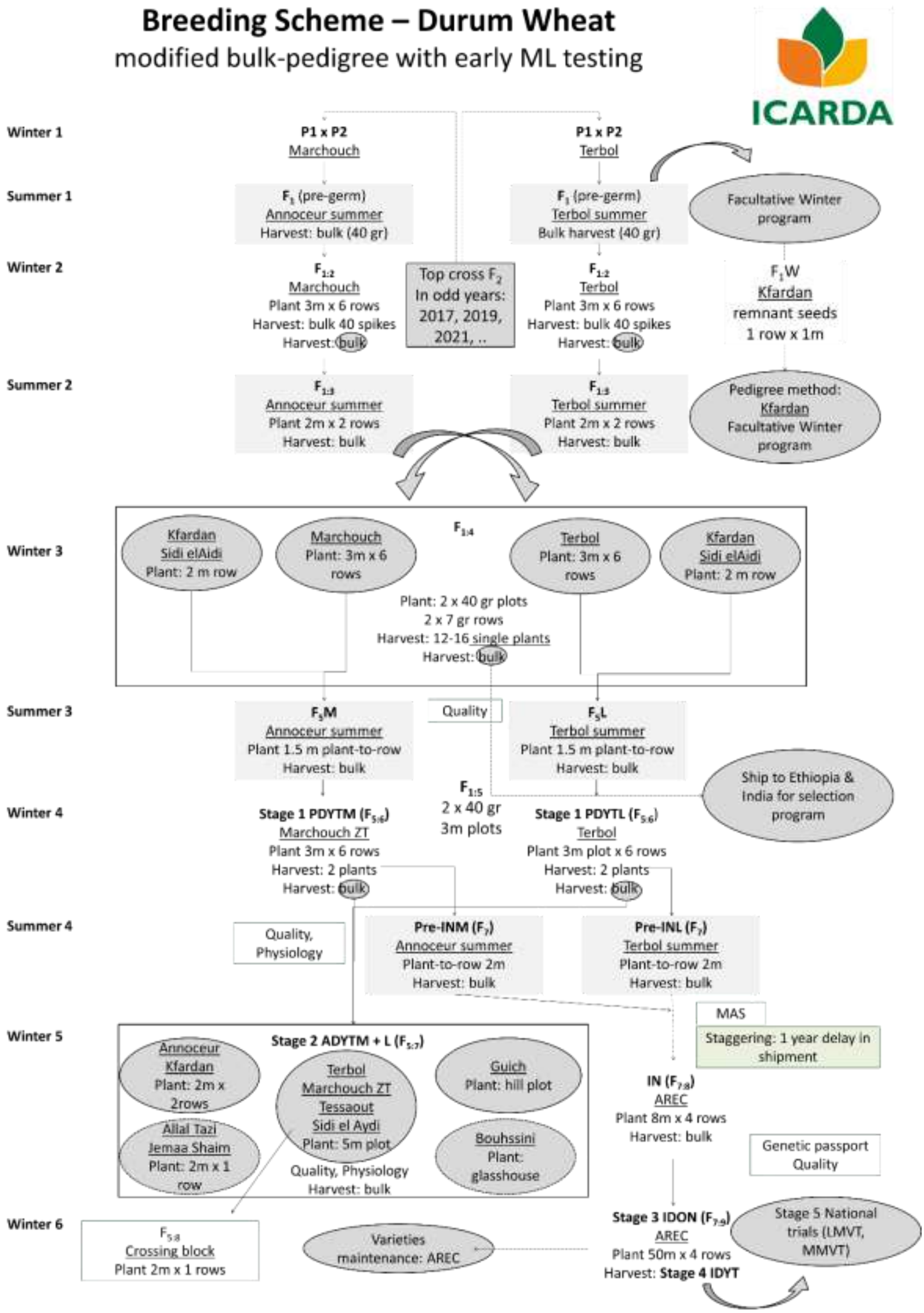


Figure 17. Breeding scheme of ICARDA durum wheat

Setting up the crossing block

A total of 15 important traits have been defined by ICARDA's partners.

The traits that need to be present in all entries:
Yield potential, 1,000 kernels weight, stability, and yellow pigment
Secondary traits:
Gluten strength, drought tolerance, low yellow berry, leaf rust, stem rust, and yellow rust resistance
PP-specific traits:
Septoria tritici, root rot, and Hessian fly resistance, heat and frost tolerance.

The crossing block (CB) needs to provide a good variation for all these traits, in order of their importance. Approximately 100 entries are assembled each year in the crossing block. These include:

- 20 lines selected from Stage 2 trials based on the highest *PP matching score*
- 15 lines from Stage 3 trials based on the highest *PP matching score*
- 10 lines from Stage 4 trials based on the highest *PP matching score*
- Five outstanding lines remaining from the previous year CB
- Five outstanding lines remaining from two years previous CB
- 10 cultivars from the targeted environments to increase adaptation
- 10 cultivars from other breeding programs (especially Canada, Europe, and Australia)
- 10 lines from CIMMYT Stage 3 trials (IDSN) for increasing diversity
- 15 entries selected from the various pre-breeding activities

Each CB entry needs to be scored by the PP matching score indicating its most suitable PP, or their specific trait of interest should be listed. A description of the main traits should also be included to simplify the decisions to be made about the crosses. A description of the lacking traits for the PP should also be included.

For the selected bulk method, a total of **500 crosses** need to be designed. The aim is to produce 2,000 F_2 seeds from each cross, which requires at least 50 F_1 plants. Considering an average of 40 seeds per F_1 plant grown during summer (three spikes x plant x 10-20 seeds). Each emasculation/pollination produces on average 10 F_1 seeds, therefore **six emasculations** are required per cross. Top crosses are made in F_2 in odd years. Each CB is planted at three different planting dates (15 November, 5 December, and 25 December) to maximize the chances of pairing phenology. Slightly different CBs are made for Lebanon and Morocco**.** These are coded as **CBL-19** or **CBM-19,** based on the year (i.e. 2019). Different PPs are addressed by stations in the two countries and therefore different crosses are made in each site.

GS and MAS crosses are described in **$F_{1:4}$ MAS** and **$F_{1:4}$ GS.** For speed-breeding by SSD, a total of **100 crosses** need to be designed. The aim is to produce 48 F_2 seeds from each cross, which requires at least four F_1 plants. Considering an average of 20 seeds per F_1 plant grown in the glasshouse (two spikes x plant x 10 seeds). Each emasculation/pollination produces on average 10 F_1 seeds, therefore **two emasculations** are required per cross. Top crosses are made in F_1. Each CB is planted in the glasshouse in Mexico at two planting dates to maximize the chances of pairing phenology. A single CB is made each year and shipped to Mexico**.** This is coded as **CBICD-19,** based on the year (i.e. 2019).

CWR-derived crosses

Special attention is attributed by the program to the use of wild relatives to introgress new useful alleles. To date, the program has released 8% of its cultivars from CWR crosses and 30% from landraces (Fig 18). In particular, the two most recent releases for Morocco (Faraj and Nachit), and the first two heat tolerant cultivars for the Senegal River (Haby and Ammina), are all CWR-derived.

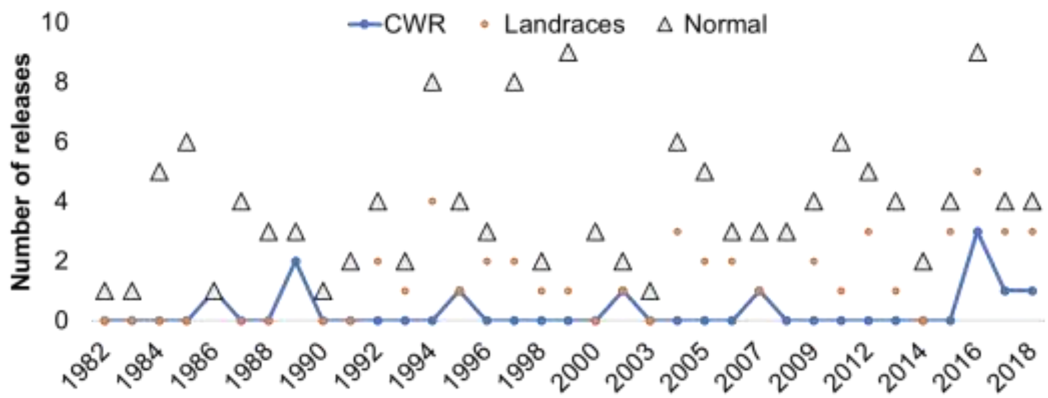


Fig 18. Pedigree origin of ICARDA's releases

In Figure 19, it is possible to notice how the top crosses made by the genebank between wild relatives and elites could deliver yield-superior, advanced lines when tested at the Stage 1 and Stage 2 at the Marchouch station. In 2017, Zaïm et al., published the article Wide Crosses of Durum Wheat Reveal Good Disease Resistance, Yield Stability, and Industrial Quality across Mediterranean Sites to express the appreciation that the program has shown for this germplasm type.

Genotyping with a 35K single nucleotide polymorphisms (SNPs) breeder chip revealed that the crosses of 'landrace x elite' produce the maximum genetic diversity, while the use of CWR tends to fix the least number of alleles (Fig 20). However, looking at unique and preferential alleles across germplasm types (Table 14), it is possible to notice that CWR-derived elites contribute 29% of the unique alleles available within the germplasm, and account for the increase in frequency of 18% of the alleles preferentially carried by one germplasm type. Since the test set included 40 elite x elite and only 21 CWR-derived, it is possible to conclude that these contribute to an increase in genetic diversity and potential for new alleles. However, the SNPs used here were developed to identify polymorphism among elites. Therefore, it will be necessary to test the same germplasm using SNPs designed on CWR alleles to better understand what portion of the CWR genome is still present in the advanced germplasm.

A comparison of 20 top crosses (A/CWR//B) revealed that 9-15% of the genome of the resulting progenies is in fact contributed by the CWR parent (Noureddine et al., 2020).

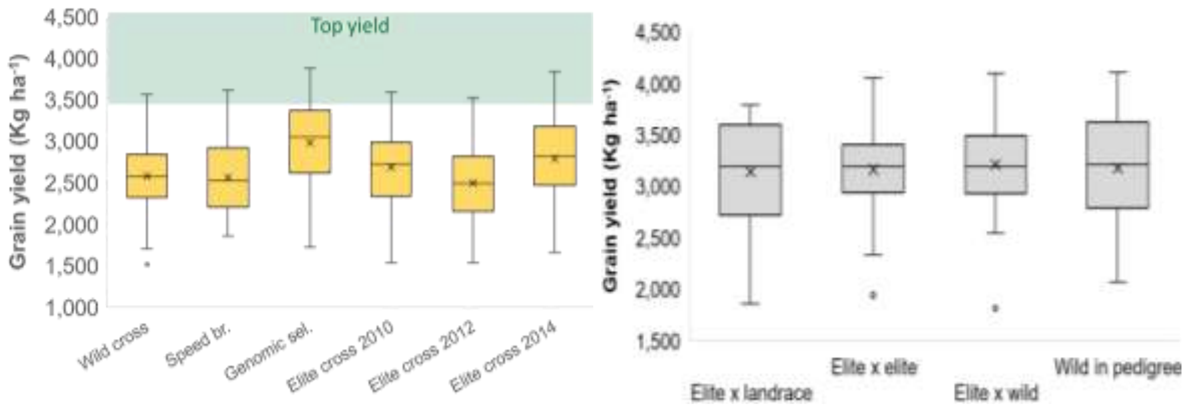


Figure 19. Yield comparison of different crossing strategies tested in Marchouch at Stage 1 trial in 2016-17 season (left) and Stage 2 in 2018-19 season (right)

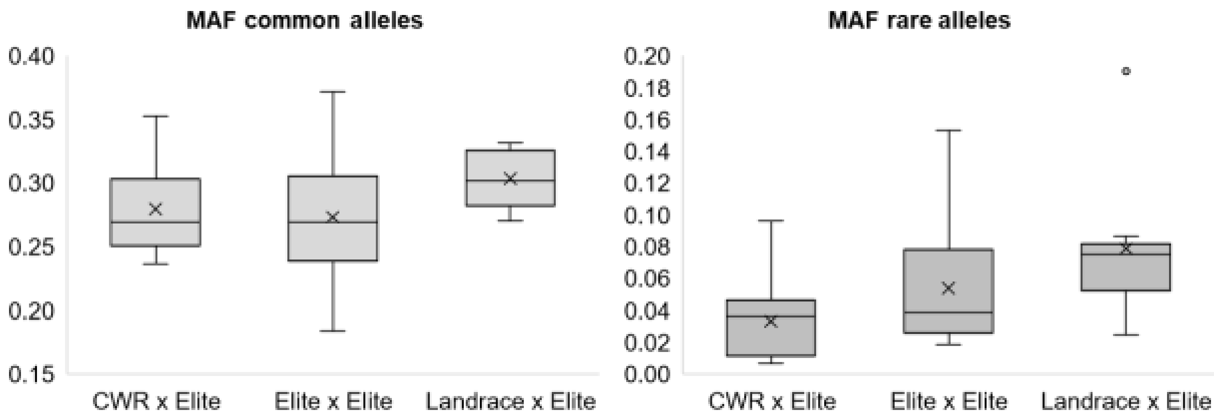


Figure 20. Minor allele frequency (MAF) of Stage 4 elite germplasm distributed at international nurseries, genotyped with 6.1K SNPs with MAF of 0.5-0.1 and 3.7K SNPs with MAF 0.1-0.01, 21 CWR x elites, 40 elite x elite, and 9 landrace x elite

Table 14. Unique minor alleles harbored by each cross type among Stage 4 elites

	CWR x elite	Elite x elite	Landrace x elite	Total
Unique alleles	229	510	60	799
	29%	64%	8%	
Preferential allele	1,100	4,978	59	6,137
	18%	81%	1%	

Annual hybridization to be carried out

Even year crosses (2020, 2022, 2024, ...)
30 crosses for each PP for a total of 300

- 10 crosses for each of the PPs need to be designed: Stage 2 x Stage 3
- 10 crosses for each of the PPs need to be designed: Stage 2 x Stage 2
- Five crosses for each of the PPs need to be designed: Stage 2 x cultivars
- Five crosses for each of the PPs need to be designed: Stage 2 x trait-specific entries

200 crosses for program improvement across PP

- 10 crosses between the top: Stage 3 x Stage 2
- 10 crosses between the top: Stage 3 x Stage 3
- 10 crosses between the top: Stage 3 x Stage 4
- 10 crosses between the top: Stage 4 x Stage 4
- 10 crosses between the top: Stage 4 x cultivars
- 10 crosses between the top: Stage 4 x CIMMYT
- 10 crosses between the top: Stage 4 x landraces
- 20 crosses between the top: Stage 4 x pre-breeding and other breeding programs

Odd years crosses (2019, 2021, 2023, ...)
300 top-crosses for each of the F_2 designed for PPs x one cross each for a total of 300 crosses

- One cross for each of the PPs need to be designed: stage 4 x F_2

200 top-crosses for each of the F_2 designed for program improvement x one cross each for a total of 300 crosses

- One cross for each of the population need to be designed: stage 4 x F_2

Every year crosses (2019, 2020, 2021, ...)
GS and MAS crosses

- 20 crosses for MAS pyramiding: two best $F_{1:4}$ per population based on MAS score x 10 populations
- 40 crosses for recurrent GS among full-sibs: 2 x 2 best $F_{1:4}$ per population based on a genomic estimated breeding value score x 10 populations

Wild crosses

- 20 crosses made by the Genebank unit: top 4 stage 4 x 5 new CWR each season

F_1L and F_1M

Early and medium
Stem rust
drought, heat, frost

A total of 500 F_1 families are generated each year, approximately 60 seeds each. These seeds are coded as F_1 followed by the country of making (i.e. F_1M or F_1L), dash "-"the year in which they will be harvested. For example, for season 2019-20 in Lebanon, these will be coded as **F_1L-20.**

Figure 21. Summer planting of nurseries in Annoceur (left) and pre-germ in Terbol (right)

Treatment for summer season F_1

The field is planted with vetch, faba bean, or field pea the previous winter. Before flowering, the crop is incorporated into the soil to increase moisture and organic matter. Before planting, 50 units of N, P, and K are provided and incorporated by cover crop. F_1 envelopes are left in the fridge at 4°C for 4 days to overcome dormancy. At the time of transplanting, one hose of abundant irrigation is provided to maximize soil moisture, and a second after 5 days. At this point, Drip pipes are installed. Irrigation is provided every 3 days for 2 hours to equal approximately 15-20 mm of moisture. Derby^{TM} and Cossack^{TM} are used as a herbicide mixture at 80% of the dose at the beginning of the season – after the appearance of the fourth leaf. Weeding is otherwise conducted by hand.

- The CB is harvested as soon as the plants reach maturity.
- 10 seeds need to be stored in the fridge in case of an unsuccessful season or realization of winter flowering behavior.
- 50 seeds per cross need to be placed in the fridge in two petri dishes (25 seeds in each) for 4 days at 4° C with a wet paper towel to promote germination and break dormancy.
- The petri dishes are then watered again and left out of the fridge for 4 days.
- The germinated seeds are directly transplanted in the summer nurseries of Annoceur or Terbol (Fig 21) as one 2.5 m row, one plant every 5-10 cm.

Stresses in F_1

- During the summer cycle in Annoceur, the plants are exposed to temperatures of up to 38° C during the day, and 16-18° C during the night. The soils are shallow and stony, with conditions that resemble severe drought. Russian wheat aphid can occur and mild natural infections of stem rust. Planting typically occurs the last week of June and harvest during the first week of October.
- During the summer cycle in Terbol the plants are exposed to temperatures of up to 36° C during the day and 16-18° C during the night. The soils are light, with conditions that resemble severe drought. Natural and artificial infections of stem rust pathotype TTTTK and TTRTF occur. Planting typically occurs the first week of July and harvest the second week of October.

Phenotypic selection in F_1

Voluntary positive selection is not conducted in F_1, however:

- Populations with mild facultative winter phenology will not flower during the summer cycle. In those cases, the remnant seeds are recovered and used the following season to create the F_1 for the facultative winter program (**F1W**).
- Populations that cannot handle the harsh conditions tend to produce fewer seeds and are less likely to be selected for the following steps. Stem rust in particular can cause severe reductions in the amount of seeds produced.
- The season is only 110-130 days from planting to harvest. As such, genotypes that flower or mature late are typically harvested when they are still green and would not produce viable seeds. As such, the biggest risk to keep in mind is that the summer season promotes earliness, which is an ideal trait for the targeted PP. However, it is necessary to consider a different approach when targeting a PP for medium or late flowering time. In this sense, CIMMYT material tends to be better adapted to later flowering conditions.

Trait selected for in F_1

Annoceur: earliness, drought and heat tolerance, mild selection pressure for stem rust and RWA.

Terbol: earliness, drought and heat tolerance, strong selection pressure for stem rust resistance.

Advancement in F_1

Annoceur: bulk harvest the whole row.

Terbol: bulk harvest the whole row.

$F_{1:2}$L and $F_{1:2}$M
Leaf rust, stem rust
drought, heat
Grain size, low yellow berry
Awnless, black awns, red straw color

Approximately 450 $F_{1:2}$ families survive the summer season. These seeds are coded as $F_{1:2}$ followed by the country of making (i.e. $F_{1:2}$M or $F_{1:2}$L), dash "-" the year in which they are harvested. For instance, seeds harvested in the 2019-20 season in Lebanon would be coded as **$F_{1:2}$L-20.**

Figure 22. Winter planting of $F_{1:2}$ in Marchouch (left) and Terbol (right)

Treatment for winter season $F_{1:2}$ in Marchouch and Terbol
In both sites (Fig 22) the soil is planted with a legume during the previous winter cycle, such as fava bean, field pea or lentil. These crops are brought to maturity and are regularly harvested.

In Marchouch, Morocco, the soils are not worked and planting is done by direct sowing (zero-till). After harvesting of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September), the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall – between the end of September and early November – it is ready for planting. Normal planting starts at around 13 November and continues until 1 December; delayed planting takes place between 1-15 December, after which planting is considered as late.

Using NPK pellets, 50 units of N, P, and K are applied using a direct seeder during planting. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (in January). Fifty units of N are provided via ammonium nitrate before a rainy day around the fifth leaf stage (January). An additional herbicide treatment is used at booting stage using a tank mixture of Mustang™ and Pallas™ (March). A further 25 units of urea are provided before heading (March). When the total in-season moisture exceeds 300 mm by the end of March, a fourth urea application of 10 units of N needs to be provided in liquid form (leaf application) after flowering (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvesting normally occurs between the end of May and middle of June.

In Terbol, Lebanon normal sowing is used. The soil is then worked with a cover crop after harvesting of the legumes. In early October, one sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts around 13 November until 5 December, delayed planting is between 5 and 20 of December and after this date is considered late planting. Using NPK pellets, the following amounts are provided: 50 units of N, P, and K and incorporated into the soil by rotary harrow.

In extreme cases when rain does not occur before the end of the planting window, irrigation of 10-15 mm is provided to support germination. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang™ and Pallas™ (April). In addition, 50 units of urea are provided before heading (April). When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July.

- The mature F_1 plants are bulk harvested from the summer season.
- The F_1 from summer Annoceur will be used for planting in winter in Marchouch.
- The F_1 from summer Terbol will be used for planting in winter in Terbol.
- 70-85 gr of seeds need to be planted in 6 m^2 plots, to ensure that 1,500-2000 plants are included (Fig 21).

Stresses in $F_{1:2}$

- During the winter cycle in Marchouch the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The soils are 0.8-1.0 m deep Vertisol of grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally with mild damage. *Septoria tritici* occurs heavily only in wet years. Leaf rust is artificially inoculated in early March via the delivery of infected plants.
- During the winter cycle in Terbol the plants are exposed to December-February night temperatures below 0° C. Irrigation prevents any occurrence of droughts, and daily temperatures rise above 30° C after flowering is completed. The soils are 0.6-0.8 m deep light Chromosol of sandy-loam. Yellow rust occurs naturally at high pressure.

Stem rust, and stem saw fly occur naturally with mild damage. Stem rust is artificially inoculated in early April via the delivery of spores on the border spreader.

Phenotypic selection in F1:2
Among-population selection is not conducted.
Within-population selection: 40 visually selected spikes are bulk harvested in $F_{1:2}$ on the basis of the following traits:

- Height: 75-100 cm.
- Phenology: early and medium-flowering types.
- Diseases: absence or weak presence of disease damage on the flag leaf.
- Agronomy: best looking spikes in terms of fertility, length, and size.
- Visual: when possible, awnless spikes or black awns will be selected.
- Physiology: preference is given to spikes with 45-80° flag leaf (vertical).

Trait selected for in $F_{1:2}$
Marchouch: earliness, height, adaptation to drought/heat, and resistance to leaf rust, TKW, low yellow berry, awns.
Terbol: earliness, height, good potential yield, and resistance to stem rust, TKW, low yellow berry, awns.

Advancement in $F_{1:2}$
Marchouch: selected bulk of 40 spikes, sow 85% of seeds of larger, low yellow berry only.
Terbol: selected bulk of 40 spikes, sow 85% of seeds of larger, low yellow berry only.

Considerations for number of spikes to be selected in $F_{1:2}$
Figure 23 shows the reported considerations for the choice of 40 spikes, equivalent to 800 seeds (20 seed each). Each PP has a target of seven main traits, and ideal phenology and height. It is assumed that the crossing parents will be fixed for many of the targeted traits (recurrent selection) since they scored high in the weighted index. Segregation for phenology and height when crossing two elites is typically reserved to two main genes each – four genes total. Disease resistance and quality characteristics are typically defined by three main genes each, for a total of two pathogens and two quality characteristics, or 12 genes per PP. One abiotic stress is included in each PP and these are controlled by four major genes each (root angle for drought, grain number for heat, winter hardiness for frost).

Stability and yield potential are important for all PPs but their control is mostly linked to minor gene interactions. Hence, for each population, at least 20 major genes need to be selected. The use of 40 bulked $F_{1:2}$ spikes ensures 92% of probability to obtain at least one plant heterozygote or homozygote for all 20 genes. The harvest of 60 spikes would be more labor-intensive and would result in limited gain. This breeding methodology does not allow effective selection for 30 or more genes.

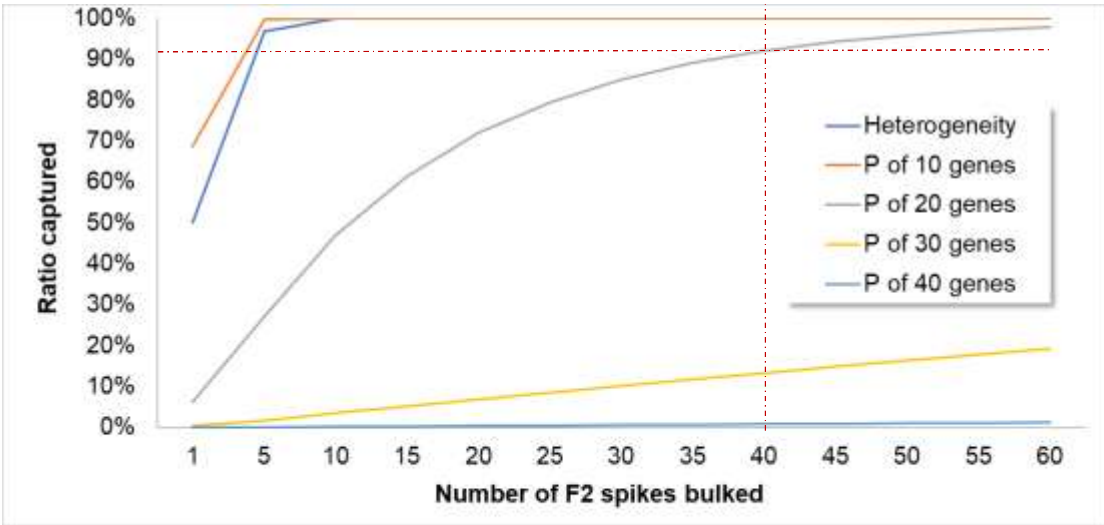


Figure 23. Effectiveness of bulked $F_{1:2}$ spikes to capture heterogeneity and select for gene combinations

P- probability of having entries with set number of genes carrying the allele in homozygous positive or heterozygous state

$F_{1:3}$L and $F_{1:3}$M

Stem rust
drought, heat

A total of 450 $F_{1:2}$ families are maintained via a bulk harvest of 40 selected spikes, approximately 50 g each. At this stage, a sieve is used to select only the 40 g of larger-sized seeds. These seeds are coded as $F_{1:3}$ followed by the country of making (i.e. M or L), dash “-“ and the year in which they will be harvested. For instance, for season 2019-20 in Lebanon these will be coded as **$F_{1:3}$L-20.**

Figure 24. Summer planting of $F_{1:3}$ nurseries in Annoceur (left) and Terbol (right)

Treatment for summer season $F_{1:3}$

The soil is planted with a legume during the winter cycle, such as faba bean, field pea or vetch. Before flowering, the crop is incorporated into the soil to increase moisture and organic matter. Before planting, 50 units of N, P, and K are provided and incorporated by a cover crop. At the time of planting, sprinkle irrigation is provided to favor germination; further irrigation is delivered after 5 days. At this point, Drip pipes are installed and used every 3 days for 2 hours to provide approximately 15 mm of moisture or 250 mm total over 6 weeks. Derby™ and Cossack™ are used as a herbicide mixture at 80% of the dose – only at the beginning of the season and after the appearance of the fourth leaf. Weeding is otherwise conducted by hand.

- The 40 spikes are harvested and bulk-threshed as soon as the plants reach maturity.
- 5 g of seeds are stored in the fridge as a backup in case of an unsuccessful season or realization of winter flowering behavior.
- 40 g of seeds per cross need to be placed in the fridge for 4 days at 4° C to break dormancy.
- The seeds are then planted in the summer nurseries of Annoceur (SAN) or Terbol (STR) (Fig 24) as two rows of 2.5 m, spaced 30 cm apart.

Stresses in $F_{1:3}$

- During the summer cycle in Annoceur, the plants are exposed to temperatures of up to 38° C during the day and 16-18° C during the night. The soils are shallow and stony, with conditions of severe drought. Russian wheat aphid can occur and mild natural infections of stem rust. Planting typically occurs during the last week of June and harvest during the first week of October.

- During the summer cycle in Terbol, the plants are exposed to temperatures of up to 36° C during the day and 16-18° C during the night. The soils are light, with conditions that resemble severe drought. Natural and artificial infections of stem rust pathotype TTTTK occur. Planting typically occurs the first week of July and harvest the second week of October.

Phenotypic selection in $F_{1:3}$

Among-population selection in $F_{1:3}$ is limited to discarding those populations that fail to flower or perform very poorly.

Within-population selection is not conducted, but the following indirect selection occurs:

- Populations with mild facultative winter phenology will not flower during the summer cycle. In those cases, the remnant seeds are recovered and used the following season to create the $F_{1:3}$ for the facultative winter program ($F_{1:3}$W).
- Populations that cannot handle the harsh winter conditions tend to produce fewer seeds and are less likely to be selected for the subsequent steps. Stem rust, in particular, can cause severe reductions in the amount of seeds produced.
- The season is only 90-110 days from planting to harvest. As such, genotypes that tend to flower or mature late are typically harvested when they are still green and therefore do not produce viable seeds. As such, the biggest risk is that the summer season promotes earliness, which is an ideal trait for the PP targeted. However, it is necessary to consider a different approach when targeting PPs for medium or late-flowering. In this case, CIMMYT material tends to be more adaptable to later flowering conditions.

Trait selected for in $F_{1:3}$

Annoceur: earliness, drought and heat tolerance, mild selection pressure for stem rust and RWA.

Terbol: earliness, drought and heat tolerance, strong selection pressure for stem rust resistance.

Advancement in $F_{1:3}$

Annoceur: bulk harvest the whole rows.

Terbol: bulk harvest the whole rows.

$F_{1:4}$L and $F_{1:4}$M

Leaf rust, stem rust, Hessian fly
Height, flowering, spike length/thickness
drought, heat
Awnless, black awns, red straw color

A total of approximately 400 $F_{1:3}$ families survive the summer season. These seeds are coded as $F_{1:4}$ followed by the country of making (i.e. $F_{1:4}$M or $F_{1:42}$L), dash "-" and the year in which they will be harvested. For instance, for season 2019-20 in Lebanon these will be coded as **$F_{1:4}$L-20**. The summer twin rows are bulk-harvested and the resulting $F_{1:4}$ seeds are split between sites.

Figure 25. Winter planting of $F_{1:4}$ in Kfardan (left) and Sidi el Aydi (right)

Treatment for winter season $F_{1:4}$ across sites
The soil is planted with a legume during the previous winter cycle, such as fava bean, field pea or lentil. These crops are brought to maturity and regularly harvested.

In Marchouch, Morocco the soils are not worked and planting is done by direct sowing (zero-till). After the harvest of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September), the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts around 13 November-1 December and delayed planting is at 1-15 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are provided by direct seeder while planting. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (January). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang™ and Pallas™ (March). Also, 25 units of urea are provided before heading (March).

When the total in-season moisture exceeds 300 mm by the end of March, a fourth urea application of 10 units of N needs to be provided in liquid form (leaf application) after flowering (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvest normally occurs between end of May and middle of June.

In Terbol, Lebanon, normal sowing is used. The soil is then worked with a cover crop after harvesting of the legumes. In early October, sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November it is ready for planting. Normal planting starts around the 13 November-5 December and delayed planting is between 5 and 20 December; after this, planting is considered as late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). In addition, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest normally occurs between the beginning of June and the middle of July.

In Sidi el Aydi, Morocco (Fig 25) normal sowing is used. The soil is worked with a cover crop using the previous season's legume harvest. In early November, after 10-20 mm of rainfall, a cover crop and disk harrow are used to kill the weeds and prepare the soil for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered as late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used as a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July. Planting is conducted with 5 g of seeds distributed in 2 rows of 2 m long plots.

In Kfardan, Lebanon (Fig 25), normal sowing is used. The soil is worked with a cover crop using the previous season's legume harvest. In early November, after 10-20 mm of rainfall, a cover crop and disk harrow are used to kill the weeds and prepare the soil for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used as a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest normally occurs between the beginning of June and the middle of July. Planting is conducted with 5 g of seeds distributed in one, 2 m row.

Stresses in $F_{1:4}$

- During the winter cycle in Marchouch, the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.8-1.0 m deep Vertisols soils are grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally, causing mild damage. *Septoria tritici* occurs heavily only in wet years. Inoculation against leaf rust is carried out artificially in early March via the delivery of infected plants.
- During the winter cycle in Terbol, the plants are exposed to December-February night temperatures of below 0° C. Irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep, light Chromosol soils are of a sandy-loam. Yellow rust occurs naturally at high pressure. Stem rust and stem saw fly occur naturally causing mild damage. Stem rust is artificially inoculated for in early April via the delivery of spores on the border spreader.
- During the winter cycle in Sidi el Aydi, the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.8 m deep Vertisol soils are of clay-loam, with several early and terminal drought occurrence. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally causing mild damage.
- During the winter cycle in Kfardan the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. Until flowering, the night temperatures often Drop below -5° C causing mild frost damage. The 0.5-0.8 m deep soils are of a sandy-clay-loam, with severe early and terminal drought occurrence. Yellow rust and stem rust occur naturally at high pressure.

Phenotypic selection in $F_{1:4}$
Among population selection: in Sidi el Aydi and Kfardan, only the best-looking populations are selected on the basis of:

- Height: 55-85 cm (height is typically reduced due to drought)
- Phenology: early-flowering types
- Drought: the naturally occurring stress is used to Drive visual selection for the best populations
- Diseases: absence or weak presence of disease damage on the row
- Hessian fly: severe damage is visible in Sidi el Aydi, and resistant populations can be selected.

In Marchouch and Terbol, the plots are also bulk-harvested and the weight is used to decide how many F_5 entries to advance to Stage 1. Harvested seeds in Marchouch are tested for NIR for several basic traits (protein, grain size, SDS and yellow pigment) and the information is used to decide how many F_5 progenies to advance per population to Stage 1.

Within population selection: the six best-looking spikes for each cross are selected in Marchouch/Terbol, and a variable number of spikes in Sidi el Aydi/Kfardan, on the basis of the following traits:

- Height: 85-100 cm (medium and tall, short types are avoided)
- Phenology: early and medium-flowering types
- Drought: the naturally occurring stress is used to impose selection
- Diseases: absence or minimal presence of disease damage on the flag leaf
- Hessian fly: severe damage is visible in Sidi el Aydi, and resistant types are selected
- Physiology: preference is given to spikes with 45-80° flag leaf (vertical)
- Agronomy: best-looking spikes in terms of fertility, length, and size, equal preference for black awns, red and white colors

Trait selected for in $F_{1:4}$
Marchouch: earliness, height, adaptation to drought/heat, biotic resistance (mainly leaf rust), and NIR test for quality
Terbol: earliness, height, good potential yield, and resistance to stem rust
Sidi el Aydi: earliness, height, adaptation to drought/heat, and biotic resistance (mainly leaf rust and Hessian fly)
Kfardan: earliness, height, adaptation to drought/heat, and biotic resistance (mainly stem rust)

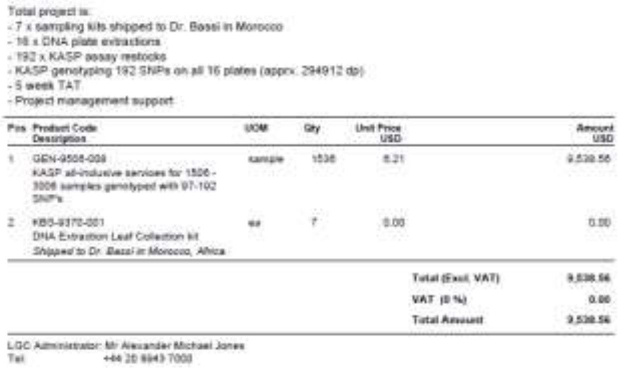
Advancement in $F_{1:4}$
Marchouch and Terbol: six individual $F_{1:4}$ plants are selected (pedigree) for each cross in each site (**F_5L** and **F_5M**). The whole plot is bulk-harvested for each cross in Terbol and used to make **ICARDA $F_{1:5}$** to be sent to Ethiopia, India and Senegal.

Sidi el Aydi and Kfardan: two-three individual $F_{1:4}$ plants are selected (pedigree) for the visually-preferred crosses (**F_5L** and **F_5M**).

$F_{1:4}$ MAS and $F_{1:4}$ GS

GS for grain size, quality characteristics, grain yield under heat and drought.
MAS for abiotic and biotic stresses, winter phenology, and quality.

A total of 20 selected $F_{1:3}$ families – 48 individuals, 4 g of seeds each – are used for glasshouse planting in cones. These are coded $F_{1:4}$ GS and $F_{1:4}$ MAS followed by, dash "-" and the year in which they will be harvested. For instance, for season 2019-20, these will be coded as **$F_{1:4}$MAS-20** and **$F_{1:4}$GS-20.**



Total project is:
- 7 x sampling kits shipped to Dr. Bassi in Morocco
- 16 x DNA plate extractions
- 192 x KASP assay restocks
- KASP genotyping 192 SNPs on all 16 plates (apprx. 294912 dp)
- 5 week TAT
- Project management support

Pos	Product Code Description	UOM	Qty	Unit Price USD	Amount USD
1	GEN-9508-008 KASP all-inclusive services for 1506 - 3008 samples genotyped with 97-192 SNP's	sample	1536	6.21	9,538.56
2	KBS-9370-001 DNA Extraction Leaf Collection kit *Shipped to Dr. Bassi in Morocco, Africa*	ea	7	0.00	0.00
				Total (Excl. VAT)	9,538.56
				VAT (0 %)	0.00
				Total Amount	9,538.56

LGC Administrator: Mr Alexander Michael Jones
Tel: +44 20 8943 7000

Figure 26. Genotyping with LGC kits and quotation for GS work

Recurrent GS strategy in $F_{1:4}$ full sibs

The complete description of the strategy for $F_{1:4}$ recurrent GS (GSRec) among full sibs can be found in:
Bassi, F.M., Bentley, A.R., Charmet, G., Ortiz, R. and Crossa, J. 2016. Breeding schemes for the implementation of GS in wheat (Triticum spp.). Plant Science, 242:23-36.

A total of 20 crosses are selected on the basis of the weighted PP index of the parents. From each, 48 individuals are pre-germinated and planted in cones (960 individuals). In addition, the Stage 2 germplasm of the previous year is also planted (480 individuals). At the fourth leaf stage, a leaf puncture is sampled in LGC Genomic KASP collection plates for full-service genotyping (DNA extraction and markers) at a cost of US$6.21 per sample (Fig 26). A total of 192 SNPs are selected as follows:

- 50 KASP: associated with known functions (QTLs or genes) for various stresses, phenology, and quality traits.
- 146 KASP: evenly distributed across the genome, with high, minor allele frequencies.

Further, the Stage 3 germplasm of each year is also planted (480 individuals). At the fourth leaf stage, leaves are collected, freeze-Dried and sent to Trait Genetics for full-service genotyping (DNA extraction and markers) with 25K array at a cost of US$32 per sample (Fig 26). The chip also includes the KASP used for low-pass genotyping.

The 25K genotyping results of Stage 3 entries are used to impute >8K SNPs, starting from the 146 KASP. These genotyping results are then used to define genetic diversity groups by conducting DAPC kinship analysis with different k:

- Populations that are somewhat similar (k=3), ideally with 10 $F_{1:4}$ populations per cluster.
- Populations that are similar (k=15), ideally with two $F_{1:4}$ populations per cluster.
- Individuals within populations that are similar (k=120), ideally with 12 $F_{1:4}$ progenies of each population per cluster.

This kinship analysis is then used to guide the crossing procedures, with hybridizations that are made:

a. Between very different populations (different assignment in k=3), but belonging to the same PP.
b. Between somewhat similar progenies (same k=3, but different k=15).
c. Between dissimilar half-sibs within population (same k=15 assignment, but different k=120).

The kinship is also used to define training populations (TP) for GS, selecting as TP the Stage 2 entries that belong to the same group (k=3) as the progenies to be predicted.

The genotyping results are then divided into two files, one for MAS and one for GS:
For MAS, the markers associated with functions are given a weight based on the selection index of each PP for each trait. The positive allele for each marker is scored as '1' and the negative as '0', and this value is multiplied by the weighted index. A MAS-weighted value is then assigned to each F_4 progeny on the basis of a simple sum of the weights for a given PP. The best F_4 family is identified as the average of the MAS weight and the best progenies within are selected. The best 10 families, four progenies each are then intercrossed within the PP considering the procedure "a" and "b" described above, to generate 20 x **F_1** for the summer cycle.

For GS, the whole set of imputed markers is used. The Stage 2 of that and previous years are used as the TP, selecting a specific TP for each progeny on the basis of kinship. A genomic estimated breeding value is then assigned to each F_4 progeny for several key traits, including quality, disease susceptibility, grain size and grain yield. These traits are then weighted using the selection index and each progeny scored for the different PP. The best F_4 families are identified as those with the highest average score and the best progenies within the family are selected. The best eight progenies for each of the top 10 families are selected and harvested. Six seeds each are planted as **F_5 GS** in the glasshouse during the same winter season (second cycle). Four plants are advanced as SSD and bulk-harvested to produce **$F_{5:6}$ GS** to be planted in the Annoceur summer season, and from there to be integrated with the normal Stage 1 trials (Fig 27). Two **F_5 GS** plants are instead used for full-sibs mating based on the procedure "c" described above, to generate 40 x **F_1 GS Recurrent 1**. Speed breeding facilities are then used to advance as SSD bulks to **F_3 GSR1** within 9 months. A total of 96 **F_4 GSR1** progenies for each population are then planted in the glasshouse and used for KASP analysis. The same markers are also used to genotype the Stage 1 trial. Imputation is repeated and four progenies per population are selected based on GS, trained by **Stage 1 GS**, which are full sibs of the progenies under GS selection. Five **F_5 GSR1** plants for each selected F_4 **GSR1** are bulk-harvested to produce **Stage 1 GSR1,** while two **F_5 GSR1** plants are instead used for full sibs mating based on the procedure "c" described above, to generate 40 x **F_1 GSR2**.

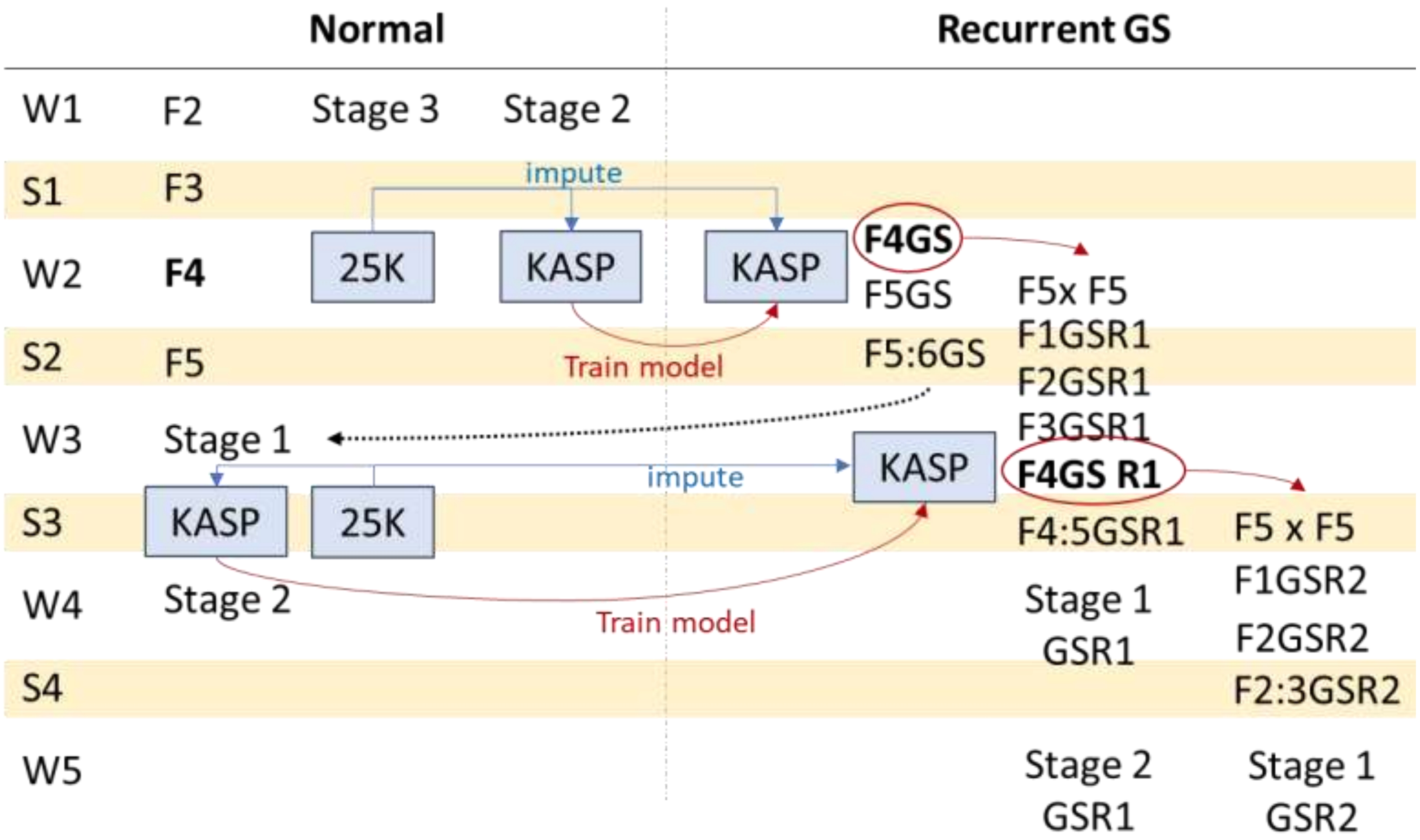


Figure 27. Schematics of genomic recurrent selection for F_4 full sibs

Comparison between normal breeding (left) and two cycles of recurrent GS between F_4 full sibs

Integrating speed breeding and SSD into molecular breeding

Leaf rust, yellow rust
MAS
GS for quality, grain size, and yield at TPE

The strategic use of speed breeding facilities associated with targeted phenotyping and deployment of markers can increase genetic gain (Fig 28). The new facilities developed by CIMMYT in El Batan, Mexico, will be used for deploying this strategy. In order to accommodate logistics and phenotyping in the speed breeding hub, seasons of 3 months (4 seasons per year) are considered.

A total of 100 F_1 families will be produced each year as follows:

Ten speed breeding crosses for 10 main PPs, totaling 100:

- Five crosses for each of the PPs need to be designed: stage 3 x stage 2
- Two crosses for each of the PPs need to be designed: stage 2 x cultivars
- Three crosses for each of the PPs need to be designed: stage 3 x trait donor

These seeds are coded as F_1 followed by a speed breeding designation "SB" to define their origin, a dash "-", and the year in which they will be harvested. For instance, for season 2020-21 in Mexico, these will be coded as **F_1SB-20.** A total of five plants per cross will be planted in pots to harvest >24 seeds per plant.

In **$F_{1:2}$SB**, 96 seeds per cross will be planted in cones. At the fourth leaf stage, a DNA sample will be collected and sent to LGC for MAS (Table 17). At the booting stage, Lr14a virulent strains of leaf rust will be inoculated to select resistant entries. The combination of leaf rust and MAS will identify the best 24 progenies for each cross. One spike each will be harvested and four seeds per spike will be moved ahead.

In **F_3SB**, 96 seeds coming from 24 F_2 spikes per cross will be planted in cones. At the booting stage, virulent strains of yellow rust will be inoculated to select 24 resistant entries from each cross. One spike each will be harvested and four seeds per spike will be moved ahead.

In **F_4SB**, 96 seeds coming from 24 F_3 spikes per cross will be planted in cones. At the fourth leaf stage, DNA samples will be collected and sent to LGC for 196 KASP genotyping, which will include 50 KASP associated to known functions (MAS) and 146 evenly spread along the genome with high PIC. The markers will be used for imputation as described above and GS models will be trained based on Stage 2 and Stage 3 yield trials. The model shall be trained to select quality characteristics, grain size, resistance to targeted diseases, and grain yield at the TPE of each PP. Selection will occur using a weighted selection index for each PP. One spike from each of the four best entries per cross will be selected and recycled to the crossing program for full sibs and half sibs mating. The best 24 entries per cross will be selected and one spike from each harvested. From each spike, two seeds will be moved ahead.

In **F_5SB**, 48 seeds coming from 24 F_4 spikes per cross will be planted in cones. No selection pressure will be imposed and a longer cycle will be used to produce sufficient seeds for the following cycle. All spikes from each plant will be harvested and shipped to Morocco.

In **F_6SB**, 48 entries per cross (4,800 entries) will be planted in one row of 1.5 m in the off-season of Annoceur. The seeds will be multiplied and used for the Stage 1 trials in the TPE the following season.

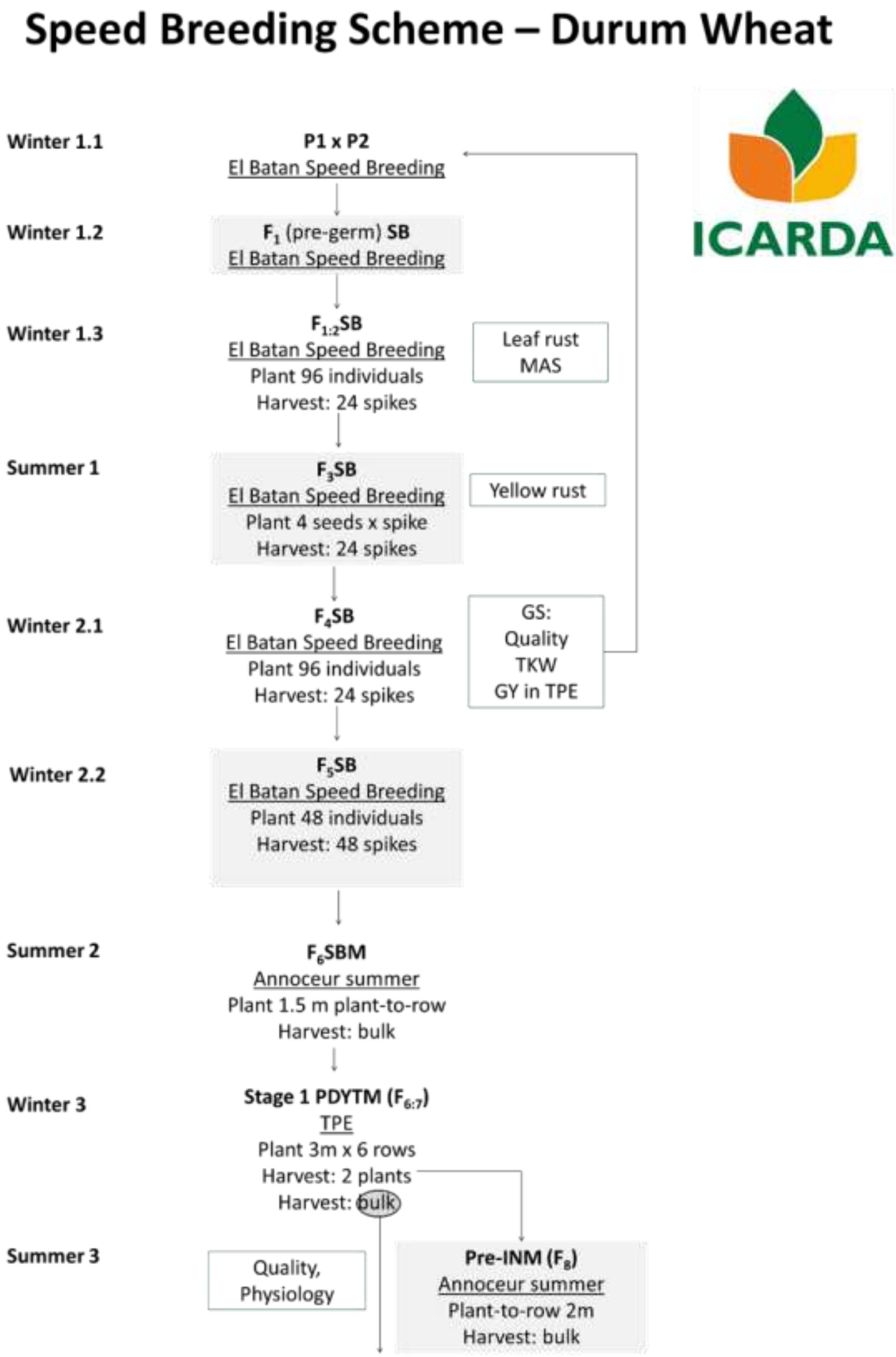


Figure 28. Breeding scheme for the deployment of molecular speed breeding

ICARDA $F_{1:5}$ for Ethiopia, India and Senegal

Each site conducts its preferred trait selection based on the PP.

A total of 200 selected $F_{1:5}$ families are obtained from bulk-harvesting of the $F_{1:4}$. For each family, 40 g each are shipped to three collaborators in Ethiopia (Debre Zeit station, Lagesse Wasihun), in India (Amalaha station, Ashutosh Sarker), and in Senegal (Fanaye station, Amadou Tidiane Sall). These are coded as **ICARDA $F_{1:5}$.**

This germplasm has been advanced since the beginning via bulking and has hence maintained the same 100% heterogeneity as the original F_1. It is thus ideal material to select germplasm best fitting the PPs that are harder to serve from the centralized locations in Morocco and Lebanon. Different selection approaches are used at each site.

Figure 29. Testing of $F_{1:5}$ in India (left) and Ethiopia (right)

Selection strategy in India, Amlaha

The growth conditions at this station are unique since very limited moisture is provided between planting and harvesting. The crop relies on moisture captured during the monsoon season by the black cotton soils (Fig 29).

The seeds are planted in a timely manner (October-early November), and one round of irrigation is provided after sowing to favor germination. One or two more irrigations are provided during the season. Individual spikes are then selected based on the overall adaptation to the conditions. F_6 spike-to-row are planted in the off-season in Wellington in collaboration with IARI-Indore, and selected based on their response to several rust pathotypes present in India. The selected rows are then harvested together, and a Stage 1 yield trial is conducted in Amalaha station as $F_{6:7}$ to select the germplasm most fitting to the specific PP.

Selection strategy in Senegal, Fanaye

The growth conditions of this station are unique since temperatures exceeding 37° C are common throughout the cycle, and the seasons are typically only 3-4 months long. The seeds are planted in a timely manner (early November-early December), and frequent, short gravity irrigations are provided every 4 days. Individual spikes are then selected based on the overall adaptation to the conditions. F_6 spike-to-row are planted during the wet season in Fanaye in collaboration with the Senegalese Institute of Agricultural Research, ISRA, and selected based on their response to severe heat. The selected rows are then harvested together, and a Stage 1 yield trial is conducted in Fanaye station the following season as $F_{6:7}$ to select the germplasm most fitting to the specific PP.

Selection strategy in Ethiopia, Debre Zeit

The growth conditions of this station are also unique since severe stem rust outbreaks occur (Fig 29). The seeds are planted in a timely manner in the off-season (early November-early December), and frequent Drip or sprinkle irrigations are provided each week. High-inoculum pressure occurs naturally during the off-season, but the seeds are further artificially inoculated to ensure no escape. Individual spikes are then selected based on their overall adaptation to the conditions. F_6 spike-to-row are planted during the normal season in Debre Zeit in collaboration with the Ethiopian Institute of Agricultural Research (EIAR), and selected again based on their response to stem rust. The selected rows are then harvested together, and a second observation nursery is conducted in the off-season at the Debre Zeit station. The best spikes are bulk-harvested and $F_{6:8}$ are used to conduct a Stage 1 yield trial at Debre Zeit and select germplasm most fitting to the specific PP.

F_5L and F_5M

Stem rust
Drought, heat

A total of 400 $F_{1:4}$ families are selected for advancement – six plants each from Marchouch and Terbol, and one-two plants from some of the families for Sidi el Aydi and Kfardan. The individual plants selected in Morocco are threshed and advanced in the Annoceur off-season, while those selected in Lebanon are advanced in the Terbol off-season. These are coded as F_5 followed by the country of making (i.e. M or L), a dash "-", and the year in which they will be harvested. For instance, for the season 2019-20 in Lebanon these will be coded as **F_5L-20.**

Figure 30. Summer planting of F_5 nurseries in Annoceur (left), stem rust inoculation (center), and Terbol (right)

Treatment for summer season F_5

The soil is planted with a legume during the winter cycle, such as faba bean, field pea or vetch. Before flowering, the crop is incorporated into the soil to increase moisture and organic matter. Before planting, 50 units of N, P, and K are provided and incorporated by a cover crop. At the time of planting, sprinkle irrigation is provided to favor germination; a second irrigation is delivered after 5 days. At this point, Drip pipes are installed and used every 3 days for 2 hours to provide approximately 15 mm of moisture or 250 mm total over 6 weeks. Derby™ and Cossack™ are used as herbicide mixture at 80% of the dose – only at the beginning of the season and after the appearance of the fourth leaf. Weeding is otherwise conducted by hand.

- The selected F_4 plants are pulled from the ground and threshed as soon as the plants reach maturity.
- 5-7 g of seeds are stored in the fridge for 4 days at 4° C to break dormancy.
- The seeds are then planted in the summer nurseries of Annoceur (SAN) or Terbol (STR) (Fig 30) as one row of 1.5 m, spaced at 30 cm.

Stresses in F_5

- During the summer cycle in Annoceur the plants are exposed to temperatures of up to 38° C during the day and 16-18° C during the night. The soils are shallow and stony, with conditions that resemble severe drought. Russian wheat aphid can occur as well as mild natural infections of stem rust. Planting typically occurs in the last week of June and harvest during the first week of October.
- During the summer cycle in Terbol the plants are exposed to temperatures of up to 36° C during the day and 16-18° C during the night. The soils are light, with conditions that resemble severe drought. Natural and artificial infections of stem rust pathotype TTTTK occur. Planting typically occurs during the first week of July and harvest during the second week of October.

Phenotypic selection in F_5

Among-population selection is limited to discarding those populations that succumb to stem rust or perform very poorly.

- The season is only 110-130 days from planting to harvest. As such, genotypes that tend to flower or mature late are typically harvested when they are still green and will not produce viable seeds. As such, the biggest risk is that the summer season promotes earliness, which is an ideal trait for the PP targeted. However, it is necessary to consider a different approach when targeting PPs for medium or late-flowering. In this case, CIMMYT material tends to be more adaptable to later-flowering conditions.

Trait selected for in F_5

Annoceur: earliness, drought and heat tolerance, mild selection pressure for stem rust and RWA.

Terbol: earliness, drought and heat tolerance, strong selection pressure for stem rust resistance.

Advancement in F_5

Annoceur: bulk-harvest the whole row.

Terbol: bulk-harvest the whole row.

Stage 1 ($F_{5:6}$) yield trials, former PDYTM and PDYTL

Height, flowering, spike number, biomass, spike length/thickness
Drought, leaf rust
Awnless, black awns, red straw color
TKW, protein content, grain color, whole flour color, SDS

A total of approximately 1,500 individual F_5 progenies survive the summer season from each off-season. These seeds are $F_{5:6}$ and used to be coded as preliminary durum yield trials (**PDYT**). Since 2019, to homogenize ontology across CG centers, the code has been changed to **Stage 1 yield trial**, followed by the country of making (i.e. Stage1M or Stage1L), a dash "-" and the year in which they will be harvested. For instance, for season 2019-20 in Lebanon these will be coded as **Stage1L-20.**

Figure 31. Winter planting of Stage 1 trials in Marchouch (left) and Terbol (right)

Treatment for winter season Stage 1

The soil is planted with a legume during the previous winter cycle, such as fava bean, lentil, or field pea. These crops are brought to maturity and regularly harvested.

In Marchouch, Morocco, the soils are not worked and planting is done by direct sowing (zero-till). After harvesting of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September) the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall – between the end of September and early November – it is ready for planting. Normal planting starts at around 13 November and continues until 1 December; delayed planting takes place between 1-15 December, after which, planting is considered as late.

Using NPK pellets, 50 units of N, P, and K are applied using a direct seeder during planting. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (in January). Fifty units of N are provided via ammonium nitrate before a rainy day around the fifth leaf stage (January). An additional herbicide treatment is used at booting stage using a tank mixture of Mustang and Pallas (March). A further 25 units of urea are provided before heading (March). When the total in-season moisture exceeds 300 mm by the end of March, a fourth urea application of 10 units of N needs to be provided in liquid form (leaf application) after flowering (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvesting normally occurs between the end of May and middle of June.

In Terbol, Lebanon normal sowing is used. The soil is then worked with a cover crop after harvest of the legumes. In early October, one sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts around 13 November until 5 December, delayed planting is between 5 and 20 of December, after which, planting is considered as late. Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow.

In extreme cases, when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to make the germination more uniform. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April). When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate but not before early April. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 35-65 g of seeds distributed in six rows of 4 m of continuous planting, then reduced to 3 m in length.*

- The plots are scored for NDVI to predict biomass and yield performances.
- In addition, the plots are visually selected.
- A maximum of 500 individuals from each Stage 1 station are identified based on the combined scores of the two points above.
- From the selected plots, two plants are selected from the border to represent the plot, pulled from the ground, threshed independently and used to plant Pre-INM in summer nurseries of Annoceur (SAN) or Pre-INL in summer Terbol (STR).
- The selected plots are also bulk-harvested and the weight is used to calculate yield. The harvested grains are also used for TKW and NIR assessment (GPC, SDS, and yellow color).
- Based on the yield and quality data, the worst 250 entries are dropped.
- The bulk seeds ($F_{5:7}$) are used to generate the **Stage 2M** and **Stage 2L** trials.

Stresses in Stage 1

- During the winter cycle in Marchouch, the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.8-1.0 m deep Vertisol are grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally, causing mild damage. *Septoria tritici* occurs heavily only in wet years. Leaf rust is artificially inoculated in early March via the delivery of infected plants.

- During the winter cycle in Terbol the plants are exposed to December-February night temperatures of below 0° C. Irrigation prevents any occurrence of drought and daily temperatures rise above 30° C after flowering is completed. The soils are 0.6-0.8 m deep, light Chromosol of sandy-loam. Yellow rust occurs naturally at high pressure. Stem rust and stem saw fly occur naturally causing mild damage. Stem rust is artificially inoculated for in early April via the delivery of spores on the border spreader.

Phenotypic selection in Stage 1
Among and within-population visual selection in Marchouch and Terbol:

- Height: 85-100 cm.
- Phenology: early and medium-flowering types.
- Drought: the naturally-occurring stress is used to impose selection.
- Diseases: absence or minimal presence of diseases is used to discard plots.
- Physiology: it is planned to add tools for scoring biomass and canopy temperature.
- Agronomy: best-looking plots in terms of biomass, number of spikes per m^2 and spike size, with equal preference for black awns, red and white colors. The selected plots are combine harvested, and the weight of the clean grains used to calculate yield performances.
- Quality: the harvested grains are used to measure TKW, NIR, grain and whole flour yellow color (b'), and SDS.
- During the slow months of the quality lab (December-March), the best entries are also milled to semolina and used to measure the yellow color (b') and the mixograph score.

Five hundred plots are visually selected at each Stage 1 site, and harvested by combine. From each visually-selected plot, two plants are pulled and threshed for off-season purification.

Trait selected for Stage 1
Marchouch: earliness, height, grain size, grain yield, adaptation to drought/heat, biotic resistance (mainly leaf rust), rheological quality.
Terbol: earliness, height, grain size, grain yield, and resistance to stem rust.

Advancement in Stage 1
Marchouch: 500 (1/3) plots are visually selected and harvested by combine. Two plants are harvested from each visually-selected plot to generate Pre-INM (F_7) to be planted in the summer off-season. The yield data, grain size (TKW), gluten index (GPC x SDS), and yellow color of whole flour are used to reduce the selection to just 230 individuals, and the bulked grains ($F_{5:7}$) are used to prepare Stage 2M.

Terbol*:* 500 (1/3) plots are visually selected and harvested by combine. Two plants are harvested from each visually selected plot to generate Pre-INM (F_7) to be planted in the summer off-season. The yield data, grain size (TKW), and visual grain score are used to reduce the selection to just 230 individuals, and the bulked grains ($F_{5:7}$) are used to prepare Stage 2L.

The strategic use of Stage 1 trials

The same families but different individuals are selected independently from joint $F_{1:4}$ trials in Lebanon at Terbol and Kfardan, and in Morocco at Marchouch and Sidi el Aydi. These individuals are advanced through the off-season as F_5 without strong selection. The use of selected bulk ensures that the large diversity captured within the 40 bulked spikes in F_2 is advanced with limited selection pressure to F_4, followed by a pedigree step with >12 progenies per population that are yield tested in Stage 1 trials. Ideally, different populations assigned to different PPs should each be Stage 1 yield tested at the station that best expresses its respective TPE. However, this would be logistically very challenging as it would require the hiring of a technical team at each main TPE farm site. Instead, the germplasm is split between Morocco and Lebanon and planted only at the main field stations in Marchouch, and Terbol.

As shown in Table 15, there is no significant correlation for grain yield among the two Stage 1 sites (r=0.12). Also, the Marchouch Stage 1 trials predict well the Stage 2 trials conducted in Morocco, while Terbol Stage 1 predicts well the Stage 2 trials in Lebanon. Therefore, the decision was made to impose a mild selection at both sites to ensure that germplasm matching all TPEs is advanced. Hence, 1/3 of the plots at each site is harvested, and 1/6 are advanced based on yields above average and acceptable rheological quality. To confirm this strategy remains effective, it is important to conduct the analysis presented in Table 15 each season.

Table 15. Correlations between Stage 1 and Stage 2 yield data

Stage 1		Stage 2					
	Marchouch vs. Terbol	Marchouch	Melk Zehr	Tessaout	Terbol	Kfardan	AREC
Marchouch	0.12	<u>0.77</u>	<u>0.72</u>	<u>0.47</u>	0.21	-0.56	-0.32
Terbol	0.12	0.34	-0.20	-0.18	<u>0.79</u>	<u>0.62</u>	<u>0.56</u>

A strategic investment in Stage 1 trials

If budget becomes available, the following interventions are suggested to increase the ratio of genetic gain:

- Increase the number of selected plants in F_4 to 12 per site instead of six in order to double the size of the Stage 1 trial.
- Increase the number of selected plots to 750 for Stage 1, and then to 500 for Stage 2 trials.
- Harvest all the Stage 1 plots instead of only those selected to determine how accurate the visual selection is.

Pre-INM (F_7) and Pre-INL (F_7)

Stem rust

Height, maturity, awns color

A total of 2,000 F_7 plants derived from 1,000 $F_{5:6}$ plots are selected for summer advancement, half from the Annoceur off-season and half from the Terbol off-season. These are coded as Pre-IN (International Nurseries) followed by the country of making (i.e. M or L), a dash "-" and the year in which they will be harvested. For instance, for the season 2019-20 in Lebanon, these will be coded as **Pre-INL-20.**

Figure 32. Summer planting of Pre-IN nurseries in Annoceur (left), and Terbol (right)

Treatment for summer season Pre-IN

The soil is planted with a legume during the winter cycle, such as fava bean, field pea or vetch. Before flowering, the legume crop is incorporated into the soil to increase moisture and organic matter, and 50 units of N, P, and K are provided and incorporated with a cover crop. At the time of planting, sprinkle irrigation is provided to favor germination; a second irrigation is provided after 5 days. At this point, Drip pipes are installed and used every 3 days for 2 hours to provide approximately 15-20 mm of moisture. Derby™ and Cossack™ are used as a herbicide mixture at 80% of the recommended dose at the beginning of the season – after the appearance of the fourth leaf. Weeding is otherwise conducted by hand.

- The selected F_6 plants are pulled from the ground and threshed as soon as the plant reaches maturity.
- 5-7 g of seeds are placed in the fridge for 4 days at 4° C to break dormancy.
- The seeds are then planted in the summer nurseries of Annoceur (SAN) or Terbol (STR) (Fig 32) as one row of 1.5 m, spaced at 30 cm.

Stresses in Pre-IN

- During the summer cycle in Annoceur, the plants are exposed to temperatures of up to 38° C during the day and 16-18° C during the night. The soils are shallow and stony, with conditions that resemble severe drought. Russian wheat aphid can occur and mild natural infections of stem rust. Planting typically occurs during the last week of June and harvest during the 1st week of October.

- During the summer cycle in Terbol, the plants are exposed to temperatures of up to 36° C during the day and 16-18° C during the night. The soils are light, with conditions that resemble severe drought. Natural and artificial infections of stem rust pathotype TTTTK occur. Planting typically during occurs the 1st week of July and harvest during the 2nd week of October.

Phenotypic selection in Pre-IN
Among the 1,000 plots selected in Stage 1 and planted in off-season as Pre-IN, only 460 plots are actually advanced to Stage 2 once agronomic and quality data become available. Hence, 540 plots x 2 individuals = 1,080 rows, which are planted in the off-season but will be discarded without further selection.

Among the remaining 460 plots x 2 individuals = 920 rows, visual selection is imposed to select only the best one of the two individuals for further use, so 460 rows total. Within-population selection of one row is conducted as follows:

- The season is only 110-130 days from planting to harvest. As such, genotypes that tend to flower or mature late are typically harvested whilst they are still green and thus, will not produce viable seeds. As such, the biggest risk is that the summer season promotes earliness, which is an ideal trait for the PP targeted. However, it is necessary to consider a different approach when targeting PPs for medium or late-flowering. In this sense, CIMMYT material tends to be more adaptable to later flowering conditions.
- Black or red awns color is preferred.
- The individual most susceptible to diseases is discarded.
- Typically, this selection pressure allows to discriminate individuals only in 60% of the plots, whilst for the others, there is no evident difference and hence both are advanced until selection can be imposed in the following step.

Trait selected for in Pre-IN
Annoceur: earliness, drought and heat tolerance, mild selection pressure for stem rust and RWA.
Terbol: earliness, drought and heat tolerance, strong selection pressure for stem rust resistance.

Advancement in Pre-IN
Annoceur: bulk harvest the whole row to generate **INM ($F_{7:8}$).**
Terbol: bulk harvest the whole row to generate **INL ($F_{7:8}$).**

Stage 2 ($F_{5:7}$) yield trials, former ADYTM and ADYTL

Height, flowering, spike number, biomass, spike length/thickness, lodging
Drought, heat, cold, yield potential
Leaf rust, yellow rust, stem rust, *Septoria tritici*, crown rot, Hessian fly, stem sawfly
Awnless, black awns, red straw color
TKW, protein content, grain color, whole flour color, SDS

A total of 460 plots from 3,000 Stage 1 plots are advanced to Stage 2 on the basis of visual selection, yield and rheological qualities. These represent, on average, 300 families originally developed via hybridization. In addition, 10 checks for different PPs are included in this trial. The experimental designs are two x alpha lattice of 240 plots (230 elites + 10 checks from Stage 5), replicated twice, with a sub-block size of six. The seeds are $F_{5:7}$ and used to be coded as advanced durum yield trials (ADYT). Since 2019, to homogenize ontology across CG centers, the code has been changed to **Stage 2 yield trial**, followed by the country of making (i.e. Stage2M or Stage2L), a dash "-" and the year in which they will be harvested. For instance, for the season 2019-20 in Lebanon, these will be coded as **Stage2L-20.**

Figure 33. Stage 2 trials
Disease screening in Allal Tazi (top left), yield testing in Tessaout (top center), fertigation test in Melk Zehr (top right), disease screening in Guich (bottom left), twin rows yield trials in Jeemaa Shaim (bottom center), and twin rows trials in Annoceur (bottom right).

Selection of Stage 2 test sites
While Stage 1 tests are meant primarily to discard germplasm with low chances of success, the Stage 2 trials are the step at which the 80 top entries that will serve all PPs are selected. It is therefore critical to select field stations that represent all TPEs and that, when combined, provide scores for all the traits of interest for the PPs.

Testing within the target agro-ecologies of the different PPs is conducted by the national partners in Stage 3 and Stage 4; the same germplasm is also tested at all possible Stage 2 sites by ICARDA. Hence, yield and climatic data of Stage 4 sites are gathered across years and used to define the TPE and how these can be represented by the possible Stage 2 stations. In Figure 10, and in previous sections of this document, the TPE was defined by GGE what sites should be used for the following season Stage 2 testing. This information was further compared with the heritabilities recorded for each station (Table 8) to use only sites with heritabilities > 0.2. The TPE definition is hence critical for the program and, each year, the GGE relationships are further studied based on Stage 4 data to improve the ability to select the best Stage 2 sites.

Figure 10 also shows how the sites selected for 2018 covered all the quadrants. However, some weakness was identified in the negative x negative (bottom left) quadrant and, for that reason, Kfardan in Lebanon was included in 2019, while the other sites were confirmed for 2019. The correlations among Stage 2 entries tested at different sites in the 2018-19 season are presented in Table 16, which confirms limited similarities between the Stage 2 stations selected, with the following exceptions:

- As expected, Marchouch matched itself in 2018 and 2019, as did Terbol in 2018 and 2019.
- Annoceur 19 correlated significantly ($r=0.32$, $p<0.01$) with Sidi el Aydi 19, and negatively to Kfardan 19 and Tessaout 19. The similarities between the two Moroccan stations represent an issue for the 2018-19 season, since Annoceur was intended to select specifically for facultative winter types for a different PP. The overlap is probably due to the extreme drought experienced by both stations in 2019, and a very mild winter occurring at the mountain station of Annoceur. The bi-plot (Fig 34) shows a trend of germplasm performing similarly across the two stations. Therefore, a decision was taken to avoid using Annoceur in the 2019-20 season for Stage 2 trials in favor of Kfardan.
- The only remaining issue was the significant correlation ($r=0.24$, $p<0.01$) between Tessaout 19 in Morocco and Terbol 19 in Lebanon. Both stations have high yields thanks to the access to irrigation water, but one is an oasis-type environment, and the other is located 1,200 m above sea level with cold winters. Looking at the bi-plot (Fig 34), it is possible to see how their interactions are mostly due to the high yield levels, rather than an actual trend in germplasm performance. As such, this interaction is deemed spurious and was not used to drive decisions for the next season.

Table 16. Correlations between stations based on Stage 2 yield data for the 2018-19 season

N=488, r=0.23 for p<0.01	AREC 19	Kfardan 19	Marchouch 18	Marchouch 19	Sidi el Aydi 19	Terbol 18	Terbol 19	Tessaout 19
Annoceur 19	0.19	**-0.32**	-0.05	0.06	**0.32**	0.02	-0.02	**-0.25**
AREC 19		-0.17	-0.05	0.09	0.02	0.15	0.05	-0.19
Kfardan 19			0.02	-0.17	**-0.24**	0.04	0.20	**0.28**
Marchouch 18				**0.77**	0.19	x	0.06	0.14
Marchouch 19					0.21	0.14	0.11	0.18
Sidi el Aydi 19						-0.16	-0.08	-0.05
Terbol 18							**0.69**	0.01
Terbol 19								**0.24**

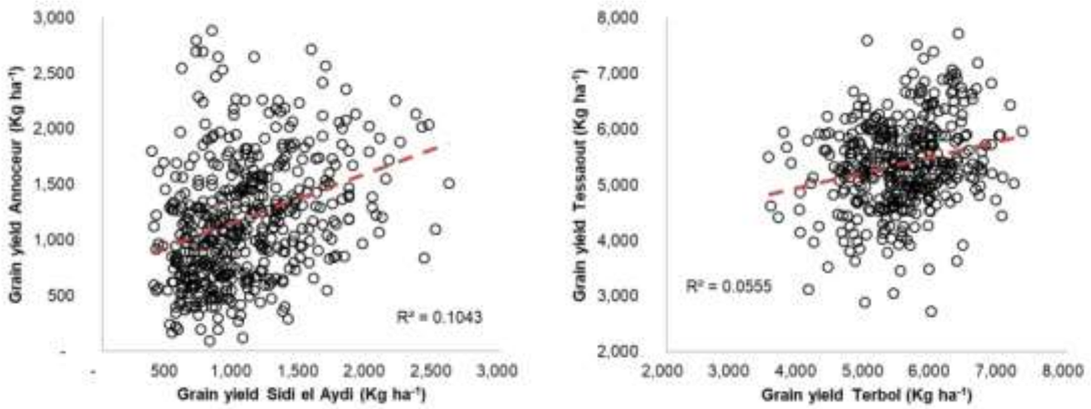


Figure 34. Bi-plot relationships of grain yield data between correlated stations

2019-20 season sites selected for Stage 2 trials

The Stage 2 yield trial testing in 7m^2 plots are conducted at:

1. Marchouch ZT, Morocco (top right quarter)
2. Terbol, Lebanon (top left quarter)
3. Sidi el Aydi, Morocco (top right quarter) – official drought platform
4. Tessaout, Morocco (bottom right quarter)

The Stage 2 yield trial testing in 2.2 m^2 plots are conducted at:

1. Kfardan, Lebanon (bottom left quarter)
2. Melk Zehr, Morocco (project ends this season, it will be Dropped next season)

The Stage 2 observation testing in 0.6 m^2 plots of 2019 are conducted at:

1. Jemaa Shaim, Morocco (Hessian fly)
2. Allal Tazi, Morocco (leaf rust, Septoria)

3. Guich, Morocco (artificial inoculation: leaf rust, Septoria)
4. Entomology, Morocco (Hessian fly)

Treatment for winter season Stage 2 across sites
In Marchouch, Morocco, the soils are not worked, and planting is carried out by direct sowing (zero-till). After harvesting of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September), the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts around 13 November-1 December and delayed planting is at 1-15 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are provided by direct seeder while planting. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (January). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (March). Also, 25 units of urea are provided before heading (March).

When the total in-season moisture exceeds 300 mm by the end of March, a fourth urea application of 10 units of N needs to be provided in liquid form (leaf application) after flowering (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvest occurs normally between end of May and middle of June. *Planting is conducted with 1,800 seeds distributed in six rows of 6 m continuous planting, which is then reduced to 5 m in length.*

In Terbol, Lebanon normal sowing is used. The soil is then worked with a cover crop after harvesting of the legumes. In early October, one sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November it is ready for planting. Normal planting starts around the 13 November-5 December and delayed planting is between 5 and 20 December; after this, planting is considered as late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used within a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest normally occurs between the beginning of June and the middle of July. *Planting is conducted with 1,800 seeds distributed in six rows of 6 m long continuous planting, which is then reduced to 5 m in length.*

In Sidi el Aydi, Morocco normal sowing is used. The soil is worked with a cover crop using the previous season's legume harvest. In early November, after 10-20 mm of rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare the soil for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used in a mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July. Planting is conducted with 1,800 seeds distributed into six, 5 m rows.

In Tessaout, Morocco (Fig 33) normal sowing is used. The soil is worked with a cover crop after a clean fallow or legume season. Normal planting starts around 20 November-5 December and delayed planting at 5-20 December; after this date, planting is considered late.

Fifty units of N, P, and K are incorporated into the soil by rotary harrow. The station receives less than 80 mm of in-season rainfall and hence productivity is ensured via gravity irrigations: 20 mm of water is provided once a week until early May to provide 550 mm in total. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate in February, and an additional 50 units of N are provided by urea in early April. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Harvest occurs normally between the beginning of June and the middle of July. Planting is conducted with 1,800 seeds distributed into six, 5 m rows.

In Kfardan, Lebanon normal sowing is used. The soil is worked with a cover crop after harvesting of the legumes or a clean fallow season. In early November, after 10-20 mm of rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare the soil for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used in a mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 400 of seeds distributed into two, 2m rows.*

In Annoceur, Morocco (Fig 33) normal sowing is used. The soil is worked with a cover crop after harvest of the legumes or clean fallow season. In late October, after 10-20 mm rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. Normal planting starts around 1-25 November and delayed planting at between 25 November and 5 December; after this date, planting is considered late.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Herbicides are used in a mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July. Planting is conducted with 400 seeds distributed into two, 2 m rows.

In Melk Zehr, Morocco (Fig 33) normal sowing is used. The soil is worked with a cover crop after harvesting of the legumes or a clean fallow season. In late October, after 10-20 mm rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. Normal planting starts around 10-25 November and delayed planting is between 25 November and 5 December; after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives less than 110 mm of in-season moisture and thus, productivity is ensured via fertigation. Irrigations of 15 mm are provided every 4 days to provide an estimated 450 mm total moisture. N is provided in 20 units via the Drippers every 15 days from 1 February onward, to provide a total of 100 units. Derby™ and Cossack™ herbicides are used as a mixture after the appearance of the fourth leaf (February). Weeding is otherwise conducted by hand. Harvest occurs normally between the end of May and the middle of June. Planting is conducted with 200 of seeds distributed into two, 2 m rows; each plot receives one Dripper between the rows.

In Jemaa Shaim, Morocco (Fig 33) normal sowing is used. The soil is worked with a cover crop after a clean fallow season. In late October, after 10-20 mm rainfall, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to Hessian fly damage, delayed sowing is used with planting at 15-25 December.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. The station receives less than 200 mm of in-season moisture, so screening for Hessian fly is the main scope here, but it can be combined with severe drought evaluations, depending on the season. Weeding is conducted by hand. Harvest, when it is carried out, occurs between the end of May and the middle of June. Planting is conducted with 100 of seeds distributed into one, 2 m row.

In Allal Tazi, Morocco (Fig 33) normal sowing is used. The soil is worked with a cover crop after a legume season. In late October, after 10-20 mm rainfall, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to the attack of leaf rust and *Septoria tritici*, normal sowing time is preferred with planting conducted between 1 and 15 December.

Using pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of the disease. In addition, spreader rows of susceptible checks are planted around and within the trials. A mixture of Derby™ and Cossack™ herbicides are used after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainfall in March. Weeding is otherwise conducted by hand. Harvesting is not carried out here. Planting is conducted with 100 of seeds distributed into one, 2 m row.

In Guich, Morocco (Fig 33) normal sowing is used. Continuous wheat planting is carried out here to maximize the disease pressure in the soil, but spikes are cut down before maturity to avoid issues with volunteers. The soil is worked with a cover crop after a legume season. In late November, after 10-20 mm rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to the attack of leaf rust and *Septoria tritici*, delayed sowing time is preferred with planting conducted between 25 and 30 December.

Using pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of the disease. In addition, spreader rows of susceptible checks are planted around and within the trials. A mixture Derby™ and Cossack™ herbicides are applied after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the rains in March. Weeding is otherwise conducted by hand. Harvest is not carried out here. Planting is conducted with four seeds that are distributed on a hill plot, and spaced at 0.3 m. In addition, 20 seeds are provided to the entomology and pathology units for seedling screening of Hessian fly, leaf rust and *Septoria tritici.*

Stresses in Stage 2

- During the winter cycle in Marchouch the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.8-1.0 m deep Vertisols soils are grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally, causing mild damage. *Septoria tritici* occurs heavily only in wet years. Leaf rust is artificially inoculated in early March via the delivery of infected plants.
- During the winter cycle in Terbol the plants are exposed to December-February night temperatures of below 0° C. Irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep, light Chromosol soils are of a sandy-loam. Yellow rust occurs naturally at high pressure. Stem rust and stem saw fly occur naturally causing mild damage. Stem rust is artificially inoculated for in early April via the delivery of spores on the border spreader.
- During the winter cycle in Sidi el Aydi the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.8 m deep Vertisol soils are of clay-loam, with several early and terminal drought occurrence. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally causing mild damage.
- During the winter cycle in Tessaout the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The e 0.9-1.0 m deep Vertisol soils are of clay-loam, with abundant moisture to ensure the expression of high yield potential. Yellow rust and leaf rust occur naturally at high pressure.
- During the winter cycle in Kfardan the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. Until flowering, the night temperatures often Drop below -5° C causing mild frost damage. The 0.5-0.8 m deep soils are of a sandy-clay-loam, with severe early and terminal drought occurrence. Yellow rust and stem rust occur naturally at high pressure.
- During the winter cycle in Melk Zehr the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.7 m deep soils are of light sand, with abundant moisture and fertilization is provided via Drip irrigation to ensure the expression of high yield potential. Yellow rust, tan spot, and leaf rust occur naturally at high pressure.
- During the winter cycle in Jemaa Shaim the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.7-0.9 m deep Vertisol soils are of clay-loam, with severe early and terminal drought occurrence. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally at very high pressure when delayed planting is used.
- During the winter cycle in Allal Tazi the plants are not exposed to any severe abiotic stresses. The 0.9-1.0 m deep soil are of clay-loam, with abundant moisture provided by gravity irrigation to ensure fungal occurrence, including by some of the world's most virulent strains of leaf rust and *Septoria tritici*. The germplasm is also exposed

to abundant moisture in difficult-to-penetrate hard soils, which favors selection against lodging.

- During the winter cycle in Guich the plants are not exposed to any severe abiotic stresses. The 0.5-0.7 m soils are of deep sand, with abundant moisture provided by rainfall and additional mist irrigation to ensure fungal attacks. Artificial inoculation against some of the world's most virulent strains of leaf rust, *Septoria tritici*, and *Fusarium culmorum* is provided.
- Seedling screening is conducted for Hessian fly, leaf rust, *Septoria tritici* and *Fusarium culmorum*.

Phenotypic selection in Stage 2

Among- and within-population selection is conducted combining data across sites:

- Quality: the harvested grains from Marchouch and Tessaout are used to measure TKW, NIR, grain and whole flour yellow color (b*), and SDS. The best entries are also milled to semolina and used to measure the yellow color (b') and the mixograph score.
- All data across sites are used to conduct statistical analyses as follows:
    - Yield, TKW, PLH, DtH, Spk.m^2, and quality lab data are estimated as BLUPs for each site, including their respective heritabilities. Scores below a set threshold of h^2 are dropped.
    - For each entry, the ratio to the best (max) genotype for each trait for each site is calculated. The average of this ratio is defined as the overall value.
    - Stability of each entry is calculated using the AWAI applied to grain yield as defined in Bassi & Sanchez-Garcia, 2017 (doi: 10.2135/cropsci2016.11.0916).
    - For the biotic scores, the worst value across tests is considered, and this is also expressed as a ratio-to-the-max.
- The selection indexes (presented in Table 10) are applied to the ratio-to-the max values to assign each entry to a specific PP.
- In addition, the yields of the station better representing the targeted TPE are used to further rank the germplasm.
- In addition, descriptive traits are also used to define the fitting of one entry to one PP. These include the phenology, the height, and the ideal awns color/type.

Trait selected for in Stage 2

All traits are weighted based on the selection index.

Advancement in Stage 2

Each PP has a different level of priority. Highly prioritized PPs will advance five entries to Stage 3, while medium priority PPs will advance three, and low priority, just one. In total, 80 entries will need to be selected for Stage 3 trials. Those that remain will be considered for addition to the crossing block.

- The top 20 entries based on the selection index score for a given PP will be ranked based on the yield performances at the station that best represents the TPE. Among these, five, three or two entries that also matching the descriptive traits will be selected.

- These selected lines will be advanced to Stage 3, but not using the $F_{5:7}$ seeds of the Stage 2 trials, but rather the $F_{7:8}$ seeds produced by Pre-IN (see next section).
    - A report will be made to show the advantage that these entries constitute for each PP compared to the PP commercial checks included in the trial.
    - The Stage 2 $F_{5:7}$ seeds of the selected entries will instead be incorporated in the crossing block for the following season.
- The entries not selected for advancement will also be considered for inclusion in the crossing block:
    - One line with the top score for the selection index of a PP, but that did not match the yield or descriptive characteristics will also be added.
    - One line with the top yield in the targeted TPE, but that had a low score for the selection index or descriptive characteristics will be added.
    - One line will also be added for each PP if it carries an important trait that is rare in the germplasm, for instance specific resistances.
    - The consideration for addition on the crossing black will also be based on the pedigree of these entries. Ideally, germplasm that has a pedigree different to those already advanced to Stage 3 will always be preferred.

INM and INL

Homogeneity
Yield and TKW at AREC
MAS

A total of 460 plant-to-row seeds are selected from Pre-INM and Pre-INL and advanced. These represent the 460 entries that are simultaneously tested as Stage 2. These seeds are $F_{7:8}$ and are coded as international nursery multiplications, followed by the country of making (i.e. INM or INL), a dash "-" and the year in which they will be harvested. For instance, for the 2019-20 season in Lebanon these will be coded as **INL-20.**

Figure 35. Winter planting of INL multiplications in AREC

Treatment for winter season IN

In AREC, Lebanon (Fig 35) normal sowing is used. The soil is worked with a cover crop after harvesting of the legumes. In early October, one boom irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts at around 13 November-5 December and delayed planting between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used as a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before rainfall is received at around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 350 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by machine. Two boom irrigations are provided in March and April. Harvest occurs normally between the beginning of June and the middle of July.

Planting is conducted with 40-80 g of seeds that are distributed into four rows spaced at 25 cm in 7 m long plots.

- The plots are visually selected for homogeneity. When evident mixture is visible, the entire plot is discarded.
- The weight of the plots and TKW are also used to decide which, among two sister lines, should be advanced to Stage 3.

Stresses in IN

- During the winter cycle in AREC the best conditions are provided to avoid stresses. The plants are exposed in December-February to night temperatures below 0° C. Mild irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep soils are light Chromosol of sandy-loam. Yellow rust occurs naturally at high pressure.

Phenotypic selection in IN

All entries are genotyped using KASP. The list of markers used for MAS are provided afterwards. For among-sister line selection in AREC:

- Visual scoring for homogeneity is performed before conducting any purification. Entries that show mixture >1% are discarded.
- Yield and TKW performances at AREC are also used to select one sister line over the other.

For among-population selection in AREC:

- The selection of which entries to promote to Stage 3 IDON is done as explained in Stage 2 trials, without using the field data from AREC, only MAS information.

Trait selected for in IN

AREC: homogeneity and MAS

MAS in IN

The LGC Genomic Complete KASP service including DNA extraction is used at a cost of US$5.47 per sample when ordering 700 samples x 25-48 markers, with a turnaround time of 6 weeks. The sampling plates are sent to Lebanon for collecting small disks of leaf tissue, and then shipped to LGC for DNA extraction and genotyping. The list of markers changes each season, depending on new validations conducted in house or from the literature. Also, markers that do not work are regularly discarded or replaced. Table 17 summarizes the status as of July 2019.

To be noted: all reviewers of durum wheat articles have agreed to impose to all authors to convert any and all QTL discoveries into validated KASP markers for ease of use in breeding.

Table 17. List of KASP markers for MAS updated as of July 2019

Marker ID	QTL/gene	Trait targeted	FAM SNP	VIC SNP	Freq positive allele
Nearly fixed in germplasm, ensure not to lose them					
wMAS000003	Lr34	Lr34	A:A	T:T (Shallow roots)	99%
AX-94385320	QTL.ICD.Ppd.002	Phenology	T:T (Insensitive)	C:C (Sensitive)	96%
BS00009709	QTL.ICD.RL3.001	Drought	C:C (Deep roots)	T:T (surface)	91%
wMAS000020	Tsn1	Tsn1	-:- (Res)	G:G (Susc)	90%
usw222	Lr_Geromtel/Tunsyr	LR	G:G (Res)	A:A (Susc)	87%
usw215	Lr_Geromtel Tunsyr	LR	G:G (Res)	T:T (Susc)	80%
usw218	Lr_Geromtel/Tunsyr	LR	G:G (Res)	A:A (Susc)	79%
AX-95260810	QTL.ICD.PlstTun.GY.006	Heat	G:G (Heat tol)	C:C (Heat susc)	72%
AX-94432276	QTL.ICD.PlstTun.GY.006	Heat	T:T (Heat tol)	C:C (Heat susc)	72%
AX-95182463	QTL.ICD.PlstTun.GY.007	Heat	C:C (Heat tol)	-:- (Heat susc)	72%
ALPa4A_285_SNP	ALPa-4A	Quality	A:A (Spitfire-type)	G:G (CS-type)	69%
IWB66138	Ei1	Biotic	G:G (Res)	A:A (Susc)	68%
GTM08_A3	Lpx-A3	Quality	T:T (Not yellow)	C:C (Yellow)	66%
BS00022785	Ei1	Biotic	C:C (Res)	T:T (Susc)	65%
Tdurum_contig82236_117	QTL nematodes	Nematodes	T:T (Res)	C:C (Susc)	64%
CIMwMAS0054	Lr14a	LR	T:T (Lr14a+)	A:A (Lr14a-)	58%
AX-95176186	QTL.ICD.RA.006	Drought	G:G (Deep roots)	-:- (Shallow roots)	57%
Cre8_SNP	Cre8	Nematodes	G:G (Res)	C:C (Susc)	54%
Achieving good fixation, continue to invest					
AX-94383178	QTL.ICD.RA.011	Drought	T:T (Deep roots)	C:C (Shallow roots)	53%
TA004946-0577	ISBW3-BM-QTL-CANDIDATE	Adaptation	C:C (Higher Biomass)	T:T (Lower Biomass)	36%
wri61	Psy1-A1l	Quality	C:C (yellow)	A:A (not yellow)	26%

Table 17. cont.

AX-94681091	QTL.ICD.PlstTun.GY.003	Heat	T:T (Heat tol)	C:C (Heat susc)	21%
AX-94679648	QTL.ICD.GYheat.004	Heat	A:A (Heat tol)	G:G (Heat susc)	18%
CIMwMAS0107	Glu-B1	Quality	G:G (Weak)	C (Bx7OE)	17%
BS00062676	Yr57	Yr57	G:G (Res)	A:A (Susc)	17%
Sr2_ger9 3p	Sr2	Sr2	C:C (Res)	- :- (Susc)	15%
Low fixation needs better targeting in CB					
BS00072387	HFAraraticum	HF	A:A (Res)	G:G (Susc)	9%
CIMwMAS0273	LrAW2/Lr61	LR	-:- (Res)	T:T=C:C=T:C (Susc)	5%
AX-94622179	QTL.ICD.HSI-GY.007	Heat	T:T (Heat tol)	C:C (Heat susc)	4%
ALPb4A_773_SNP	ALPb-4A	Quality	G:G (good)	A:A (bad)	3%
CIMwMAS0135	Lr19/Sr25	LR SR	C:C (Susc)	T:T (Res)	1%
Not useful or not working					
CIMwMAS0028	Rht-B1_SNP	Phenology	G (RhtB1_SNP-AL1)	A (RhtB1_SNP-AL2)	99%
GTM02_c	Lpx-B1.1b	Quality	G:G (New cultivar)	A:A (Old cultivar)	99%
AX-94733268	QTL.ICD.RL2.001	Drought	G:G (Drought tol)	T:T (Drought susc	98%
AX-94932858	QTL.ICD.HSI-GY.003	Heat	A:A (Heat tol)	T:T (Heat susc	94%
AX-94549122	QTL.ICD.HSI-GY.002	Heat	C:C (Heat tol)	T:T (Heat susc	84%
Tdurum_contig10380_87	QTL nematodes	Nematodes	T:T (Res)	C:C (Susceptible	81%
AX-94509297	QTL.ICD.HSI-TKW.002	Heat	C:C (Heat tol)	A:A (Heat susc	79%
BS00022364	QTL.ICD.GYKFD17A.001	Drought	A:A (Drought tol)	G:G (Drought susc	76%
AX-95115092	QTL.ICD.AWAI.007	Stability	A:A (Stable)	G:G (not stable	76%
BS00004224	QTL.ICD.PlstTun.TKW.001	Agronomic	C:C (Large grains)	T:T (Small garins)	63%
AX-95140644	QTL.ICD.Vrn.007	Phenology	G:G (Early)	-:- (Late)	23%
AX-95213349	QTL.ICD.Vrn.006	Phenology	C:C (Early)	T:T (Late)	3%
CIMwMAS0055	Lr47-1	LR	C:C (Lr47-1_AL1)	G:G (Lr47-1_AL2)	0%
CIMwMAS0255	Sr22_A_AL-Sus-T	SR	T:T (Al1)	A:A (Al2)	0%

Advancement from IN multiplication to Stage 3 IDON by staggering

AREC: Exactly 80 entries are advanced each year from Stage 2 to Stage 3 as described in the previous section. The seeds for the Stage 3 test (international nurseries) are generated in the IN multiplications at AREC, Lebanon. The Stage 3 nurseries are typically shared with 40-50 partners from 20-30 countries each year. As such, the preparation and dispatch of seeds requires several weeks. Further, all seeds need to be tested by the Seed Health Lab. Finally, the full yield, MAS, pathology, and quality data only became available at the end of July, which leaves less than 1 week for statistical analysis and selection. For this reason, starting from 2019 (IDON 43rd), the breeding program has adopted a strategy of staggering the sets, meaning that the Stage 3 selection for the 2019-20 season will be shipped and tested only in 2021-22, skipping one season. Unfortunately, this will have a negative impact on the genetic gain, since 1 year is added to the cycle, which is why it is critical to recycle the germplasm for the crossing block already at Stage 2 testing, where staggering does not occur.

Stage 3 International durum observation nursery – (IDON)

Genetic passport

Homogeneity

Yield and TKW at TPE

A total of 96 $F_{7:9}$ plots are shipped as part of the IDON to partners around the world. The IDON is set up as a spatial augmented design with four sub-blocks of size 24, each incorporating three checks (Omrabi5, Waha, and Miki 3). It provides 83 new entries from ICARDA, three checks present four times, and one empty slot for incorporating a national check by each partner. The set is coded as IDON, dash "-", followed by a progressive number which started from the first shipment in 1977. For instance, for the season 2019-20, the code is **IDON-43rd.** The IDON set can be ordered from ICARDA's international nursery website: https://indms.icarda.org/

On average, 40-50 sets are provided each year to 20-30 countries (Fig 36) based on requests and availability of seeds. The IDON are cultivated in three different formats: one set of 400 seeds each entry to be planted in twin rows of 2 m; a test set used by ICARDA of 1,800 seeds planted as a plot in Marchouch and Terbol; and 350 g for 40 m long multiplication in AREC.

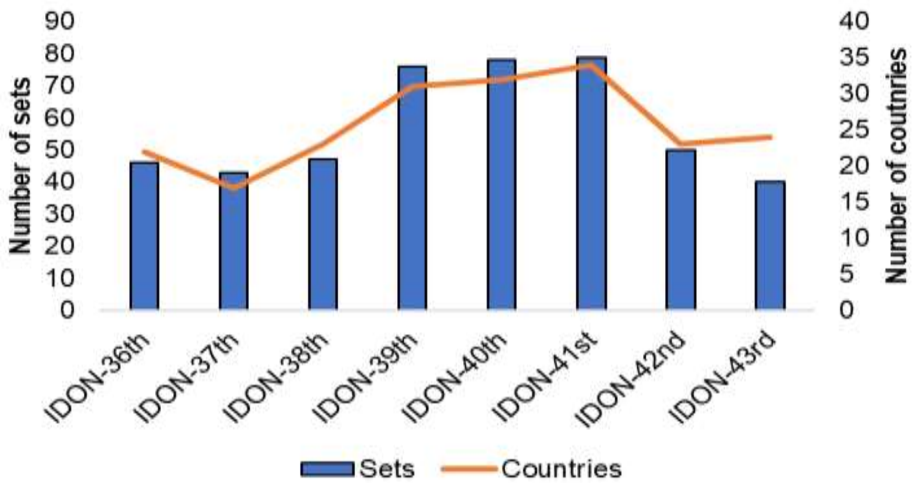


Figure 36. Winter planting of IDON multiplications in AREC, plot evaluation in Marchouch, and past 8 years of IDON distribution

Treatment for winter season Stage 3 IDON
In AREC, Lebanon (Fig 36) normal sowing is used. The soil is worked with a cover crop after harvesting of the legumes. In early October, one boom irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts at around 13 November-5 December and delayed planting between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used as a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before rainfall is received at around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 350 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by machine. Two boom irrigations are provided in March and April. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 40-80 g of seeds distributed into four, 40 m long rows, spaced at 30 cm.*

In Marchouch, Morocco the soils are not worked and planting is done by direct sowing (zero-till) (Fig 36). After the harvest of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September) the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts around 13 November-1 December and delayed planting is at 1-15 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are provided by direct seeder while planting. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (January). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (March). Also, 25 units of urea are provided before heading (March).

When the total in-season moisture exceeds 300 mm by the end of March, a fourth urea application of 10 units of N needs to be provided in liquid form (leaf application) after flowering (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvest occurs normally between end of May and middle of June. *Planting is conducted with 1,800 seeds distributed into six, 6 m row of continuous planting, which are then reduced to 5 m in length.*

In Terbol, Lebanon normal sowing is used. The soil is then worked with a cover crop after harvesting of the legumes. In early October, one sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November it is ready for planting. Normal planting starts around the 13 November-5 December and delayed planting is between 5 and 20 December; after this, planting is considered as late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used within a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest normally occurs between the beginning of June and the middle of July. *Planting is conducted with 1,800 seeds distributed to in six, 6 m rows of continuous planting, which are then reduced to 5 m in length.*

In Allal Tazi, Morocco normal sowing is used. The soil is worked with a cover crop after a legume season. In late October, after 10-20 mm rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to the attack of leaf rust and *Septoria tritici*, normal sowing time is preferred with planting conducted between 1 and 15 December.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of disease. In addition, spreader rows of susceptible checks are planted around and within the trials. Derby™ and Cossack™ are used within a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the rains in March. Weeding is otherwise conducted by hand. Harvest is not carried out here. *Planting is conducted with 100 seeds distributed in one, 2 m row.*

In Guich, Morocco normal sowing is used. Continuous wheat planting is carried out here to maximize the disease pressure in the soil, but spikes are cut down before maturity to avoid issues with volunteers. The soil is worked with a cover crop after a legumes season. In late November, after 10-20 mm rainfall a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to leaf rust and *Septoria tritici* attack, delayed sowing time is preferred with planting conducted between 25 and 30 December.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of disease. In addition, spreader rows of susceptible checks are planted around and within the trials. Derby™ and Cossack™ are used within a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the rains in March. Weeding is otherwise conducted by hand. Harvest is not carried out here. *Planting is conducted with four seeds distributed on one hill plot and spaced at 0.3 m.* In addition, 20 seeds are provided to the entomology and pathology units for seedling screening of Hessian fly, leaf rust and *Septoria tritici*.

The key to success for the IDON is not in the testing at the sites under ICARDA's control, but the data collected by partners around the world. Over 40 sets are field evaluated annually for many characters in different TPE. The data are typically recovered from approximately 40% of the tested sites depending on the partners' capacities. These results are then combined with those collected by ICARDA and used to make selections. Different planting conditions are used by each site, but all test the IDON as *400 seeds in twin rows of 2 m.*

Stresses in Stage 3 IDON

- The IDON are exposed to nearly all possible biotic and abiotic stresses by the partners' testing. Data on disease response, grain yield, phenology and TKW are obtained each year.
- During the winter cycle in AREC the best conditions are provided to avoid stresses. The plants are exposed in December-February to night temperatures below 0° C. Mild irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep light Chromosol soils are of sandy-loam. Yellow rust occurs naturally at high pressure.
- During the winter cycle in Marchouch the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.8-1.0 m deep Vertisol soils are of grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally causing mild damage. *Septoria tritici* occurs heavily only in wet years. Leaf rust is artificially inoculated in early March via the delivery of infected plants.
- During the winter cycle in Terbol the plants are exposed to December-February night temperatures below 0° C. Irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep light Chromosol soils are of sandy-loam. Yellow rust occurs naturally at high pressure. Stem rust, and stem saw fly occur naturally causing mild damage. Stem rust is artificially inoculated in early April via the delivery of spores on the border spreader.
- During the winter cycle in Sidi el Aydi the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.8 m deep Vertisol soils are of clay-loam, with several early and terminal droughts. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally causing mild-high damage.

- During the winter cycle in Tessaout the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.9-1.0 m deep Vertisol soils are of clay-loam, with abundant moisture to ensure the expression of high yield potential. Yellow rust, and leaf rust occur naturally at high pressure.
- During the winter cycle in Kfardan the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. Until flowering, the night temperatures often Drop below -5° C causing mild frost damage. The 0.5-0.8 m deep soils are sandy-clay-loam, with severe early and terminal drought occurrence. Yellow rust, and stem rust occur naturally at high pressure.
- During the winter cycle in Melk Zehr the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.7 m deep soils are of light sand, with abundant moisture and fertilization provided via Drip irrigation to ensure the expression of high yield potential. Yellow rust, tan spot and leaf rust occur naturally at high pressure.
- During the winter cycle in Jemaa Shaim the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.7-0.9 m deep Vertisol soils are of clay-loam, with severe early and terminal drought occurrence. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally at very high pressure when delayed planting is used.
- During the winter cycle in Allal Tazi the plants are not exposed to any severe abiotic stresses. The 0.9-1.0 m deep soils are of clay-loam, with abundant moisture provided by gravity irrigation to ensure fungal attack, including by some of the world's most virulent strains of leaf rust and *Septoria tritici*. The germplasm is also exposed to abundant moisture in difficult-to-penetrate hard soils, which favors selection again lodging.
- During the winter cycle in Guich the plants are not exposed to any severe abiotic stresses. The 0.5-0.7 m soils are of deep sand, with abundant moisture provided by rainfall and additional mist irrigation to ensure fungal attack. Artificial inoculation of some of the world's most virulent strains of leaf rust, *Septoria tritici*, and *Fusarium culmorum* is applied.
- Seedling screening is also conducted for Hessian fly, leaf rust, *Septoria tritici*, and *Fusarium culmorum*.

Phenotypic selection in Stage 3 IDON

All entries are genotyped using a 20K Axiom array to generate genetic the passport data. This information is stored and used to later identify the use of the germplasm by partners and to train GS models.

For homogeneity selection in AREC:

- Visual scoring for homogeneity is performed before conducting any purification. Entries that show mixture >1% are discarded.
- Germination and absence of seed-transferable diseases is also used to select the best germplasm.

Among- and within-population selection is conducted combining data across sites:

- Quality: the harvested grains from Marchouch and Sidi el Aydi are used to measure TKW, grain protein content (NIR), grain and whole flour yellow color (b'), SDS. The best entries are also milled to semolina and used to measure the yellow color (b*) and the mixograph score.
- All data across sites are used to conduct statistical analysis as follows:
    - Yield, TKW, PLH, DtH, Spk.m^2, and quality lab data are estimated as BLUPs for each site, including their respective heritabilities. Scores below a set threshold of h^2 are dropped.
    - For each entry, the ratio to the best (max) genotype for each trait for each site is calculated. The average of this ratio is defined as the overall value.
    - Stability of each entry is calculated using the AWAI applied to grain yield as defined in Bassi & Sanchez-Garcia, 2017.
    - For the biotic scores, the worst value across tests is considered, and this is also expressed as a ratio-to-the-max.
- The selection indexes (presented in Table 10) are applied to the ratio-to-the max values to assign each entry to a specific PP.
- In addition, the yields of the station better representing the targeted TPE are used to further rank the germplasm.
- In addition, descriptive traits are also used to define the fitting of one entry to one PP. These include the phenology, the height, and the ideal awns color/type.

Traits selected for in Stage 3 IDON
AREC: homogeneity and MAS
All traits are weighted based on selection index (Table 9).

Advancement in Stage 3 IDON
Each PP has a different level of priority. Highly prioritized PP will advance two entries to Stage 4, while medium priority will advance only one. In total, 20 entries will need to be selected for the Stage 4 trials; those remaining will be considered for addition to the crossing block.

- The top 20 entries based on selection index score for a given PP will be ranked based on the yield performances at the station best representing the TPE. Among these, one or two will be selected that also match the descriptive traits.
- These selected lines will be advanced to Stage 4 not using the $F_{7:9}$ seeds produced in AREC.
    - A report will be made to show the advantage that these entries constitute for each PP compared to the PP commercial checks included in the trial.
- The entries not selected for advancement will be also considered for inclusion in the crossing block:
    - One line with the top score for the selection index of a PP, but that did not match the yield or descriptive characteristics will also be added.
    - One line with the top yield in the targeted TPE, but that had a low score for the selection index or descriptive characteristics will be added.

- One line will also be added for each PP if it carries an important trait that is rare in the germplasm, for instance specific resistances.

The consideration for addition on the crossing black will also be based on the pedigree of these entries. Ideally, germplasm that has a pedigree different than those already advanced to Stage 4 will always be preferred.

Advancement from multiplication of Stage 3 to Stage 4 IDYT by staggering

AREC: Exactly 20 entries are advanced each year from Stage 3 to Stage 4 as described in the previous section. The seeds for the Stage 4 test (international nurseries) are generated from the multiplications at AREC. To avoid further delay, the decision of which entries to include in the Stage 4 IDYT is made each year, without additional staggering. This means only the data received before July of each year is used to prepare the set. However, the number of entries is sufficiently small that the automated system set up at AREC can easily prepare and dispatch the sets before the end of September.

Stage 4 IDYT

All traits at TPE

A total of 23 $F_{7:10}$ plots are shipped as part of the IDYT to partners around the World. The IDYT is set up as an alpha lattice with two replicas and four sub-blocks of size six. It includes 20 new entries, three historical checks (Omrabi5, Waha, and Miki 3), and one empty slot for the national check to be included by each partner. This set is coded as IDYT, a dash "-", following by a progressive number started from the first shipment in 1977. For instance, for the 2019-20 season, the code is **IDYT-43rd.** The IDYT set can be ordered from ICARDA's international nursery website: https://indms.icarda.org/

On average, 60-70 sets are provided each year to 20-30 countries (Fig 37) based on requests and availability of seeds. The IDYT are cultivated in two different formats: a set for partners of 1,800 seeds to be planted as a plot; and 350 g for 40 m multiplication plots in AREC.

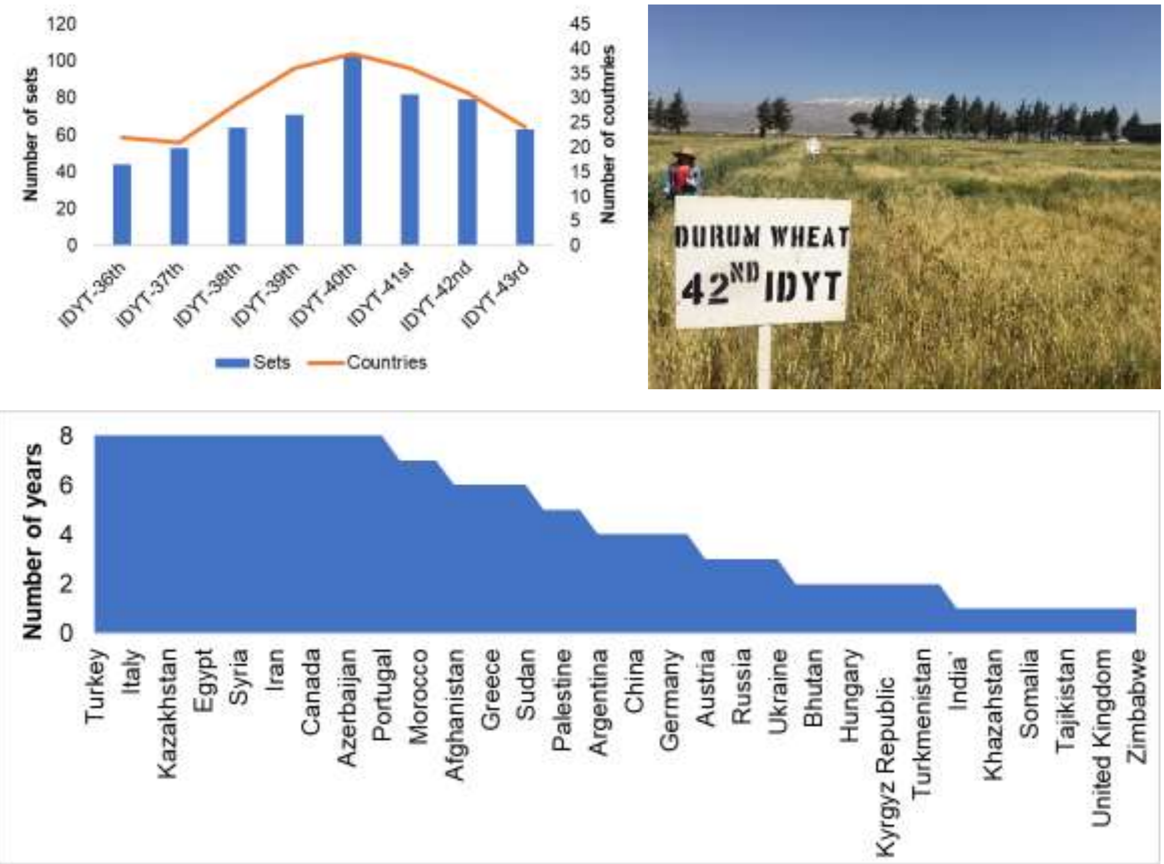


Figure 37. Past 8 years' requests of IDYT sets and countries requesting the sets over the years. Top right – IDYT test in Terbol, Lebanon

To facilitate the use of the germplasm by our NARS partners, the main trait performance of the Stage 4 elites are presented in the form of a table directly inside the field book that they receive with the seeds. In addition, each entry is provided with the code of the PP that match it, and the selection index used for the assignment is also presented (Table 18 and Table 19).

Treatment for winter season Stage 4 IDYT
In AREC, Lebanon normal sowing is used. The soil is worked with a cover crop after harvesting of the legumes. In early October, one boom irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts at around 13 November-5 December and delayed planting between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby^{TM} and Cossack^{TM} are used as a herbicide mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before rainfall is received at around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 350 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by machine. Two boom irrigations are provided in March and April. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 40-80 g of seeds distributed in 4 rows x 40 m long spaced 30 cm plots.*

In Marchouch, Morocco the soils are not worked and planting is done by direct sowing (zero-till). After the harvest of the legume, glyphosate is applied to control weeds and Orobanche. At the end of summer (September) the largest remaining weeds are removed by hand. Once the soil has received 10-20 mm of rainfall between the end of September and early November, it is ready for planting. Normal planting starts during 13 November-1 December and delayed planting at 1-15 December; planting after this date is considered late.

Using NPK pellets, 50 units of N, P, and K are provided by direct seeder while planting. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to ensure uniformity of the germination. Derby^{TM} and Cossack^{TM} herbicides are applied as a mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate before rainfall at around the fifth leaf stage (January). An additional herbicide treatment is applied at the booting stage via a tank mixture of Mustang and Pallas (March). A further 50 units of urea are provided before heading (March).

When the total in-season moisture exceeds 250 mm by the end of March, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (April).

Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early March. Harvest occurs normally between end of May and middle of June. *Planting is conducted with 1,800 seeds distributed into six rows of 6 m continuous planting, which are then reduced to 5 m in length.*

Table 18. Assignment of Stage 4 IDYT as presented to the partners in the field book received with the seeds

Name	Product profiles	Gen	GY potential	GY stability	Drought tolerance	Heat tolerance	Yellow pigment	Gluten strg	Large grains	LR resist	SR resist	HF res	ST res	Flowering	Hegiht
Bob	1, 3, 6	F7:10	●		●		○			○	○			E	S
Hoffjihan	3, 6	F7:10	○	○			○	●			○	●		E	M
Hoffmilmus	3	F7:10	○		○	●	●				○	●		L	M
Icambeljoudy1	4, 5, 14, 11	F7:10					●	●	●	○	○			M	S
Icambeljoudy2		F7:10				○		○	●	○	○			M	M
Icambelmiki	2, 4, 5, 7	F7:10	○			○		○			○		●	M	M
Icambeltagy	2	F7:10		○				●			○			M	M
Joudatlas	13	F7:10		●	○			○	○		○		○	E	T
Joudenses	12, 8, 1, 6	F7:10	●		●				●		○			E	M
Joudmiki	6	F7:10		●			●	○		○	○		●	L	T
Joudysyr	7	F7:10		●	○		●		○		○			M	M
Ouassetel	7	F7:10				○							○	M	M
Ouaverve	9, 11, 8, 1	F7:10	●	○	●		○	●			○			E	S
Ouzzjihan	6, 10	F7:10				●		○	○		○			L	T
Poeticum	7, 8	F7:10		○		○							●	M	S
Saraverves	12, 11	F7:10	●		○			●	○		○			E	M
Sebasabil	2, 12, 9, 11, 8	F7:10	○	○	●			●	●		○			M	M
Sebaverve	14	F7:10	○	○	○		○	●			○		●	L	M
Tillejihan	9	F7:10						●	○	○	○			M	T
Urammar		F7:10		●			○			●	○		○	M	M

Table 19. Selection index used for assigning each entry to a specific PP as presented to the partners inside the field book received with the seeds

Code	Country	Zone	Frost	Hessian fly	Heat	Root rot	Septoria	YR	SR	LR	yellow berry	Drought	Gluten str	Yellow pigment	Stability	TKW	Yield potential
P1	Algeria	Drylands (200-300 mm)	0%	5%	0%	5%	0%	10%	0%	10%	0%	10%	0%	10%	15%	15%	20%
P2	Algeria	High plateaus (200-300 mm)	0%	0%	0%	0%	10%	0%	0%	5%	5%	10%	10%	10%	15%	15%	20%
P3	Morocco	Unfavorable zones (200-350 mm)	0%	10%	0%	0%	0%	10%	0%	5%	0%	10%	10%	10%	10%	15%	20%
P4	Morocco	Semi-favourbale (500-400 mm)	0%	0%	0%	0%	5%	5%	5%	10%	5%	0%	10%	10%	15%	15%	20%
P5	Morocco	Semi-favourbale (500-400 mm)	0%	0%	0%	0%	15%	5%	5%	10%	5%	0%	10%	10%	10%	10%	20%
P6	Morocco	High drylands (200-400 mm)	15%	0%	0%	0%	0%	0%	0%	0%	10%	10%	0%	15%	15%	15%	20%
P7	Ethiopia	Mid-highlands (optimal moisture)	0%	0%	0%	5%	0%	10%	15%	0%	0%	0%	10%	10%	15%	15%	20%
P8	Ethiopia	Lowlands (200-300 mm)	0%	0%	0%	5%	0%	10%	10%	0%	0%	10%	5%	10%	15%	15%	20%
P9	India	Early planting, 1-2 irrigation, MP	0%	0%	0%	0%	0%	0%	10%	5%	10%	10%	10%	10%	10%	15%	20%
P10	Senegal	Hot steppe irrigated	0%	0%	20%	0%	0%	0%	0%	0%	5%	0%	10%	15%	15%	15%	20%
P11	Lebanon	Supplemental irrigation	0%	0%	0%	5%	0%	0%	10%	0%	5%	0%	15%	15%	15%	15%	20%
P12	Tunisia	Semi-arid	0%	0%	0%	10%	0%	0%	0%	5%	10%	5%	10%	10%	15%	15%	20%
P13	Tunisia	Semi-arid	0%	0%	0%	5%	0%	0%	0%	5%	10%	10%	10%	10%	15%	15%	20%
P14	Tunisia	Semi-favorable (400-600 mm)	0%	0%	0%	0%	15%	0%	5%	5%	10%	0%	5%	10%	15%	15%	20%

In Terbol, Lebanon normal sowing is used (Fig 38). The soil is then worked with a cover crop after harvesting of the legumes. In early October, one sprinkle irrigation is used to favor the germination of the remaining weeds. In late October, a cover crop and disk harrow are used to kill the weeds. Once the soil has received 10-20 mm of rainfall between the end of September and early November it is ready for planting. Normal planting starts around the 13 November-5 December and delayed planting is between 5 and 20 December; after this, planting is considered as late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ are used within a herbicide mixture after the appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before a rainy day around the fifth leaf stage (March). An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Also, 50 units of urea are provided before heading (April).

When the total in-season moisture exceeds 450 mm by the end of April, a fourth urea application needs to be provided in liquid form (leaf application) after flowering (May). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest normally occurs between the beginning of June and the middle of July. *Planting is conducted with 1,800 seeds distributed into six rows of 6 m long continuous planting, which are then reduced to 5 m in length*

In Sidi el Aydi, Morocco normal sowing is used. The soil is worked with a cover crop using the previous season's legume harvest. In early November, after 10-20 mm of rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare the soil for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used in a mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the anticipated rainfall in February. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Continuous planting is preferred, and the alleys are cut by glyphosate not before early April. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 1800 seeds distributed into six, 5 m rows.*

In Tessaout, Morocco normal sowing is used. The soil is worked with a cover crop after a clean fallow or legume season. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. The station receives less than 80 mm of in-season rainfall and hence productivity is ensured via gravity irrigations: 20 mm of water is provided once a week until early May to provide 550 mm in total. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (January). Fifty units of N are provided by ammonium nitrate in February, and an additional 50 units of N are provided by urea in early April. An additional herbicide treatment is used at the booting stage using a tank mixture of Mustang and Pallas (April). Weeding is otherwise conducted by hand. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 1,800 seeds distributed into six, 5 m rows.*

In Kfardan, Lebanon normal sowing is used. The soil is worked with a cover crop after harvest of the legumes or a clean fallow season. In early November, after 10-20 mm rainfall a cover crop and disk harrow are used to kill the weeds and prepare it for planting. Normal planting starts around 20 November-5 December and delayed planting is between 5 and 20 December; planting after this date is considered late planting.

Using NPK pellets, 50 units of N, P, and K are incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to uniform the germination. Derby™ and Cossack™ herbicides are used in a mixture after appearance of the fourth leaf (February). Fifty units of N are provided by ammonium nitrate before the rains in February. Weeding is otherwise conducted by hand. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 400 seeds distributed into two, 2 m rows.*

In Annoceur, Morocco normal sowing is used. The soil is worked with a cover crop after harvest of the legumes or a clean fallow season. In late October, after 10-20 mm rainfall has been received by the soil, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. Normal planting starts around 1-25 November, and delayed planting is between 25 November and 5 December; planting after this date is considered late.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. In extreme cases when rain does not occur before the end of the planting window, an irrigation of 10-15 mm is provided to ensure uniformity of the germination. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (February). An additional 50 units of N are provided by ammonium nitrate before the rains in February. Weeding is otherwise conducted by hand. Harvest occurs normally between the beginning of June and the middle of July. *Planting is conducted with 1,800 seeds distributed in six, 5 m rows.*

In Jemaa Shaim, Morocco normal sowing is used. The soil is worked with a cover crop after clean fallow season. In late October, after 10-20 mm rainfall a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to Hessian fly damage, delayed sowing is used and planting takes place between 15 and 25 December.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives less than 200 mm of in-season moisture, so screening for Hessian fly is the main scope here, but it can be combined with severe drought evaluations, depending on the season. Weeding is conducted by hand. Harvest, when it is carried out, occurs between the end of May and the middle of June. *Planting is conducted with 100 seeds distributed into one, 2 m row.*

In Allal Tazi, Morocco normal sowing is used. The soil is worked with a cover crop after a legume season. In late October, after 10-20 mm rainfall a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to the attack of leaf rust and *Septoria tritici*, normal sowing time is preferred with planting conducted between 1 and 15 December.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of the disease. In addition, spreader rows of susceptible checks are planted around and within the trials. Derby™ and Cossack™ are used as herbicide mixture after the appearance of the fourth leaf (February). An additional 50 units of N are provided by ammonium nitrate before the rains in March. Weeding is otherwise conducted by hand. Harvest is not carried out here. *Planting is conducted with 100 seeds distributed into one, 2 m row.*

In Guich, Morocco normal sowing is used. Continuous wheat planting is carried out here to maximize the disease pressure in the soil, but spikes are cut down before maturity to avoid issues with volunteers. The soil is worked with a cover crop after a legumes season. In late November, after 10-20 mm rainfall, a cover crop and disk harrow are used to kill the weeds and prepare it for planting. To expose the crop to the attack of leaf rust and *Septoria tritici*, delayed sowing time is preferred and planting is conducted between 25 and 30 December.

Using NPK pellets, 50 units of N, P, and K and incorporated into the soil by rotary harrow. The station receives abundant rainfall, but two additional sprinkle irrigations are provided in March and April to favor the establishment of the disease. In addition, spreader rows of susceptible checks are planted around and within the trials. Derby™ and Cossack™ are used as a herbicide mixture after the appearance of the fourth leaf (February). An additional 50 units of N are provided by ammonium nitrate before rainfall in March. Weeding is otherwise conducted by hand. Harvest is not carried out here. *Planting is conducted with 10 seeds distributed on one hill plot and spaced 0.3 m.* In addition, 20 seeds are provided to the entomology and pathology units for seedling screening of Hessian fly, leaf rust and *Septoria tritici*.

The true key for success of the IDYT is not in the testing at the sites under ICARDA's control, but the data collected by partners around the world. Over 50 sets are field evaluated annually for many characters in different TPE. The data are typically recovered from approximately 40% of the tested sites depending on the partners' capacities. These results are then combined with those collected by ICARDA and used to make selections. Different planting conditions are used by each site, but all test the IDON.

Stresses in Stage 4 IDYT

- The IDYT are exposed to nearly all possible biotic and abiotic stresses by the partners' testing.
- During the winter cycle in AREC the best conditions are provided to avoid stresses. The plants are exposed in December-February to night temperatures below 0° C. Mild irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep light Chromosol soils are of sandy-loam. Yellow rust occurs naturally at high pressure.
- During the winter cycle in Marchouch the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.8-1.0 m deep Vertisol soils are of grey loam-clays, with typical early and terminal drought occurrence. Yellow rust and tan spot occur naturally at high pressure. Leaf rust, crown/root rot, Hessian fly, and stem saw fly occur naturally causing mild damage. *Septoria tritici* occurs heavily only in wet years. Leaf rust is artificially inoculated in early March via the delivery of infected plants.
- During the winter cycle in Terbol the plants are exposed to December-February night temperatures below 0° C. Irrigation prevents any occurrence of drought, and daily temperatures rise above 30° C after flowering is completed. The 0.6-0.8 m deep light chomosol soils are of sandy-loam. Yellow rust occurs naturally at high pressure. Stem rust and stem saw fly occur naturally causing mild damage. Stem rust is artificially inoculated in early April via the delivery of spores on the border spreader.
- During the winter cycle in Sidi el Aydi the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.5-0.8 m deep Vertisol soils are of clay-loam, with several early and terminal droughts occurring. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally with mild-high damage.
- During the winter cycle in Tessaout the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.9-1.0 m deep Vertisol soils are of clay-loam, with abundant moisture to ensure the expression of high yield potential. Yellow rust and leaf rust occur naturally at high pressure.
- During the winter cycle in Kfardan the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. Until flowering, the night temperatures often Drop below -5° C causing mild frost damage. The soils are 0.5-0.8 m deep sandy-clay-loam, with severe early and terminal droughts occurrence. Yellow rust, and stem rust occur naturally at high pressure.

- During the winter cycle in Jemaa Shaim the plants are exposed to terminal temperatures of up to 35° C during the day, and above 30° C during the flowering stage. The 0.7-0.9 m deep Vertisol soils are of clay-loam, with severe early and terminal drought occurrence. Yellow rust, leaf rust and crown rot occur naturally at high pressure. Hessian fly and stem saw fly occur naturally at very high pressure when delayed planting is used.
- During the winter cycle in Allal Tazi the plants are not exposed to any severe abiotic stresses. The 0.9-1.0 m deep soils are of clay-loam, with abundant moisture provided by gravity irrigation to ensure fungal attack, including by some of the world's most virulent strains of leaf rust and *Septoria tritici*. The germplasm is also exposed to abundant moisture in difficult-to-penetrate hard soils, which favors selection again lodging.
- During the winter cycle in Guich the plants are not exposed to any severe abiotic stresses. The 0.5-0.7 m deep soils are sandy, with abundant moisture provided by rainfall and additional mist irrigation to ensure fungal attack. Artificial inoculation is also applied to some of the world's most virulent strains of leaf rust, *Septoria tritici*, and *Fusarium culmorum*.
- Seedling screening is conducted for Hessian fly, leaf rust, *Septoria tritici*, and *Fusarium culmorum*.

Phenotypic selection in Stage 4 IDYT
The data collected from IDYT are used to train the GS model, and to help select the TPE for Stage 2 testing of the following season by GGE analysis (Fig 33 and Table 16).

For homogeneity selection in AREC:
- Visual scoring for homogeneity is performed before conducting any purification. Entries that show mixture >1% are discarded.
- Germination and absence of seed-transferable diseases is also used to select the best germplasm.

Among- and within-population selection is conducted combining data across sites:
- Quality: the harvested grains from Marchouch, Tessaout, and Sidi el Aydi are used to measure TKW, grain protein content (NIR), grain and whole flour yellow color (b*), SDS. The best entries are also milled to semolina and used to measure the yellow color (b*), the mixograph score, and pasta firmness after cooking.
- All data across sites are used to conduct statistical analysis as follows:
    - Yield, TKW, PLH, DtH, Spk.m^2, and quality lab data are estimated as BLUPs for each site, including their respective heritabilities. Scores below a set threshold of h^2 are dropped.
    - For each entry, the ratio to the best (max) genotype for each trait for each site is calculated. The average of this ratio is defined as the overall value.
    - Stability of each entry is calculated using the AWAI applied to grain yield as defined in Bassi & Sanchez-Garcia, 2017.

    - For the biotic scores, the worst value across tests is considered, and this is also expressed as a ratio-to-the-max.
- The selection indexes (as presented in Table 10) are applied to the ratio-to-the max values to confirm that each entry matches the specific PP to which it was assigned.
- In addition, the yields of the station best representing the targeted TPE are used to further rank the germplasm.
- In addition, descriptive traits are also used to define the fitting of one entry to one PP. These include the phenology, the height, and the ideal awns color/type.
- The PP matching score is derived from the combinations of these values, and used to evaluate the effectiveness of the breeding program to deliver for each PP.

Traits selected for in Stage 4 IDYT
AREC: homogeneity and MAS
All traits are weighted based on selection index (Fig 14).

Advancement in Stage 4 IDYT
At this stage, the substantial majority of the ICARDA breeding program is complete, since the primary target is to deliver germplasm to national breeders. The main activity is to ensure the maintenance and production of high-purity seeds to feed into ICARDA's large scaling projects with its national partners. To ensure this:

- 12 spikes are annually harvested from each of the 20 IDYT entries to be planted the following year as G_0, and produce pure breeder's seed.

However, in some cases, bi-lateral projects are in place to work with national partners to assess the performances of the best germplasm against the best selections by national breeders and private companies. To date, this is the case for India, Lebanon, Morocco, and Senegal. For those countries:

- The top four entries based on the PP matching score for the PP requested by those countries are selected and incorporated into **Stage 5 trials.**

Breeders’ seeds G_0 to G_3

Homogeneity

Part of ICARDA’s mandate is to ensure the availability of good quantities of high-purity seeds to support those national partners that need it. This type of seed is mostly used to meet the needs of ICARDA’s scaling projects, and to support the approaches of ‘village/community-based seed enterprises’ where these are allowed.

The European definition of seeds from G_0 to G_3 is used for coding. The code is completed by indicating the trial from which these originated, a dash”-“, and the year of harvest. For instance, for IDYT42nd harvested in the 2018-19 season and planted in 2020, it would be **IDYT42nd-$G_0$20** then **IDYT42nd-$G_1$21** the following season.

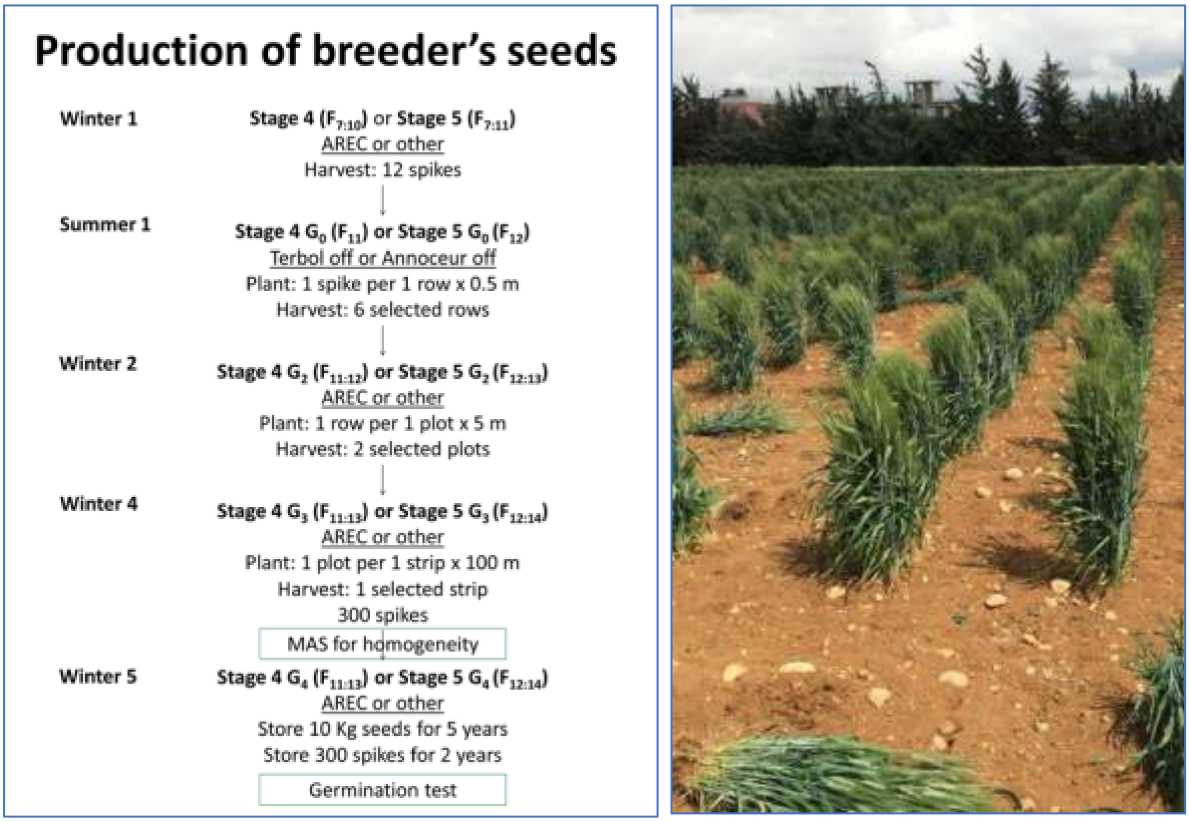


Figure 38. Approach to produce breeders’ seeds from Stage 4 or Stage 5 plots and maintenance of spike-to-row varieties in AREC

The steps involved in the production of breeders’ seeds are graphically presented in Figure 38. From the external rows of the plots of Stage 4 (IDYT) or Stage 5 (“C”MVT) trials, 12 spikes are collected from all entries in rep 1. These spikes are planted in the summer off-season as three small broken pieces per spike (no threshing) in a row of 0.5 m. Based on phenology, disease response, height and spike morphology, the six most similar spike-to-row examples are harvested independently.

During the winter season, each row is planted in one plot of 5 m in length. Plots are selected for homogeneity, and compared to each other for phenology, height, and morphology. Two plots are selected and, in the following season, are planted into a strip of 100 m in length. Leaf samples are collected from 12 randomly distributed plants along each strip and the KASP markers described in the GS chapter are used to determine heterogeneity. Moreover, the same marker is compared with the genetic passport of Stage 3 IDON to ensure that no seed mixing has occurred. From the selected strip, 300 spikes and 10 kg of seeds are harvested and stored at 4° C for 4 years to support seed certification requests. These seeds are tested to ensure >92% germination before storage.

For Stage 5 “C”MVT entries, the seeds of the discarded strip are instead used for submission to the national catalogue trial.

Stage 5 multi-location variety trials (MMVT, SMVT, IMVT, LMVT)

All traits at TPE

For those countries that have bi-lateral agreements with ICARDA, a Stage 5 'special nursery' is conducted jointly across locations. This is the case in Morocco (MMVT), Senegal River (SMVT), India MP (IMVT) and Lebanon (LMVT). Each trial is composed using the following general guideline:

- 10% widely-grown commercial cultivars
- 15% newly-released varieties
- 15% best entries selected the previous season
- 20% new ICARDA elites selected from IDYT (Stage 4) conducted at the TPE
- 20% new CIMMYT elites selected from IDYN (Stage 4) conducted at the TPE
- 20% new elites proposed by the national partner

The trial is set up as an alpha lattice with two or three replicas and sub-blocks of size six. This set is coded as "C"MVT, where "C" stands for the country (M-Morocco, S-Senegal River, I-India, L-Lebanon), followed by a dash "-" and then the year in which it will be harvested. For instance, for the 2019-20 season in Morocco, the code is **MMVT20.**

This trial provides several opportunities to:

- Walk the plots together with national breeders to identify gaps and successes (Fig 39);
- Compare in an objective format the performances of the commercial cultivars, CGIAR germplasm, and entries from the national breeders;
- Jointly identify those elites that match the different PPs;
- Promote the best entries for national catalogue registration.

Figure 39. Joint selection of MMVT19 at Tessaout station (left) and of LMVT at Kfardan station (right)

Treatment for winter season Stage 5
Each station follows different procedures to mimic the surrounding farmers' conditions and to ensure high heritabilities and low CV.

Stresses in Stage 5

- These are decided jointly with NARS partners based on the traits requested for each PP.
- The stations (TPE) for testing are selected as a consequence.
- The germplasm is exposed to the different targeted stresses.

Phenotypic selection in Stage 5
The data collected (Fig 40) are used to assign the germplasm to specific PPs (Table 20) and define the missing traits.

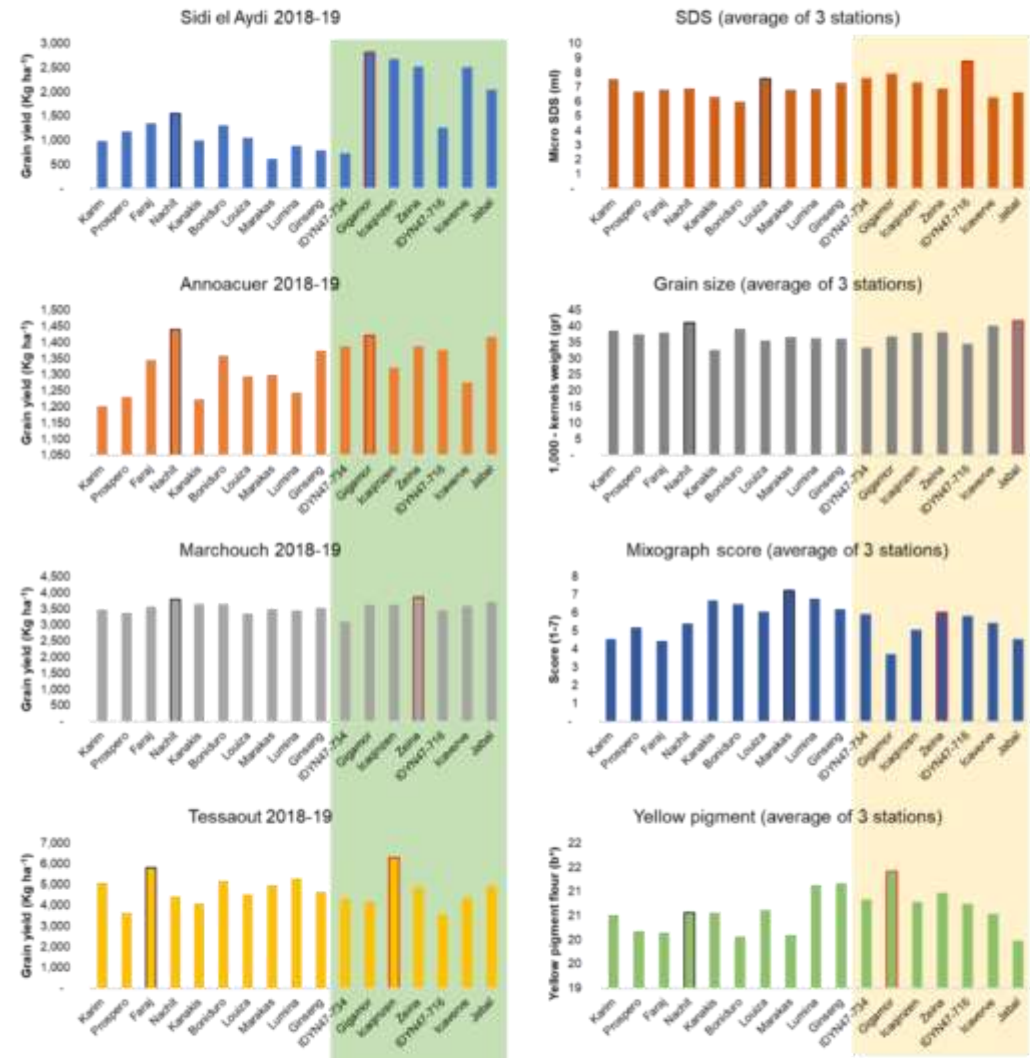


Figure 40. Phenotypic data collected for MMVT19 across sites

Table 20. Assignment of elite germplasm to four Moroccan PPs

	Adapted to	Yield gain	Phen.	Height	rgeted trai	Match P1	Match P2	Match P3	Match P4
Gigamor	High-drylands and unfavorable zones	20%	Early	75-85 cm	TKW, YP, HF, YR, LR	Good (SDS)	Poor (SDS)	Poor (SDS, ST)	Good (medium)
Zeina	Unfavorable zones	20%	Medium	80-90 cm	TKW, SDS, HF, YR, LR	Good (YP)	Poor (YP)	Poor (YP, ST)	Medium (YP)
Icaqinzen	Unfavorable & Semi-favorable	20%	Late	85-95 cm	TKW, LR, SDS, HF, YR	Medium (YP, early)	Good (YP)	Medium (YP, ST)	Bad (medium, YP)
IDYN47-734	Hih-drylands & semi-favorable	20%	Early	85-95 cm	YP, SDS, YR, LR	Poor (TKW, HF)	Good (TKW)	Medium (TKW, ST)	Medium (medium, TKW)
Icaverve	Unfavorable & Semi-favorable	20%	Medium	85-95 cm	YP, HF, YR, ST, LR	Poor (TKW, SDS, early)	Good (TKW, SDS)	Good (TKW, SDS)	Medium (TKW)
Jabal	High-drylands and unfavorable zones	20%	Early	90-100 cm	TKW, SDS, YR, LR	Poor (YP, HF)	Medium (YP)	Medium (YP, ST)	Very good (medium)

Traits in parenthesis are still lacking. Only lines that out-yielded the check in the TPE are scored as 'good' and above.
YR, yellow rust; HF, Hessian fly; LR, leaf rust; ST, stem rust.

Traits selected for in Stage 5 "C"MVT

All traits are weighted based on the selection index.

Advancement in Stage 5 "C"MVT

The germplasm that enters the "C"MVT is discarded if it does not match any PP, or is repeated the following year in "C"MVT if it displayed good performances for a given PP. The final output of "C"MVT is to jointly identify with national partners possible entries for release as varieties. These top entries are also used for producing the demonstration plots. For most of the countries, there are strict requirements to accept germplasm in the catalogue trial. The strictest is the North African system, which requires 50 kg of 98% pure seeds for trial testing and 300 spikes at 99.1% homogeneity for uniformity testing. To ensure this:

- 12 spikes are annually harvested from each of the plots to be planted the following summer season as G_0 and produce pure breeders' seed (see previous chapter).

Demo plots

Capturing farmer's preferences

As part of ICARDA's activities, it is important to be able to capture farmers' preferences as well as train farmers and extension agents. At the two main stations of ICARDA (Marchouch and Terbol), and in special TPE, demonstration plots are regularly conducted following some basic principles. The demonstration plot size changes depending on land availability and can space between long plots of six rows of 20 m, to large plots of 100 m^2, to full-scale validations of 1,000 m^2 (Fig 41).

Figure 41. Demonstration plots of 20 m in Terbol, of 100 m^2 in Melk Zehr, and 1,000 m^2 in Marchouch

Setting up the demo plots

The principle for the demonstration is to ensure to capture the farmers' preferences as well as the potential yield gain compared to the most grown cultivar. Hence, their composition is typically limited to six or 12 plots divided as follows:

Class 1: two or four plots of commercially available varieties
Class 2: two or four plots of recently released varieties
Class 3: two or four plots of new propositions for release

The new propositions are identified from Stage 5 trials as those entries that have shown at least two seasons of performances superior to the check. When any entry is released, it will continue to be included in the demo but as Class 2 instead of 3.

The order of planting follows the plan of the visit, with farmers starting with commercial varieties they already know, then the recently released ones that need to be promoted, and finally, the future releases to capture their preference/appreciation.

Capturing farmers' interest

A strategic way to capture farmers' preferences is to ask them to stand in front of the entries that they have preferred and explain to their peers why they liked it best. If farmers mostly stand in front of Class 2 and 3 plots, it means that good breeding progress was achieved. It is also critical to understand the preferred traits to incorporate these in the following season selection and in the decision of which varieties to propose to the catalogue.

Facultative winter program

In the Atlas Mountains of North Africa, some of the cooler Drylands of West Asia (especially Afghanistan, Iran and Iraq), and several regions in Central Asia, cultivars with a facultative winter phenology tend to be superior to true spring types. This is because days with a lower average temperature ensure longer seasons, which are less suitable for fast-growing spring types. In addition, winter night temperatures can often fall below -5° C, which tends to cause severe winter damage to true spring types. To serve these regions, it is important to dedicate a separate breeding effort to target these areas. However, given the overall smaller surface compared to spring durum, ICARDA dedicates only a small parallel durum program dedicated to facultative winter germplasm.

The stations of Kfardan in Lebanon experience severe winter damage associated with early and terminal droughts. Because of this combination of stresses, it is an ideal site to carry on a facultative winter program. In addition, the use of the summer off-seasons in Annoceur and Terbol allows rapid identification of any F_1 cross with vernalization requirements, since those do not flower or flower extremely late under hot summer conditions.

A schematic representation of the program is presented in Figure 42. This program feeds a special nursery defined as the International Facultative Winter Durum Observation Nursery (IFWDON), which is shipped to national partners around the world once every 5-10 years (the next is due in 2022). To achieve this nursery, the details are presented below.

F_1W

The F_1 that do not flower during the summer off-seasons are identified. The 5-10 remnant F_1 seed that were not planted in the summer off-seasons are then re-coded as F_1W (winter), dash "-", and the year of harvest. For instance, for the 2019-20 season, these would be F_1W_{20}. These seeds are then planted as a short row of 0.5 m and bulk-harvested.

$F_{1:2}W$

The F_1 seeds are bulk-harvested and planted as one row of 2 m in Kfardan station. A total of 20 spikes are selected and bulk-harvested based on their winter survival and overall appearance.

$F_{1:3}W$

The $F_{1:2}$ seeds are planted as two rows of 2 m in Kfardan station. A total of 20 spikes are selected and bulk-harvested based on their winter survival and overall appearance.

$F_{1:4}W$

The $F_{1:3}$ seeds are planted as two rows of 2 m in Kfardan station. A total of four spikes are selected from each plot based on their winter survival and overall appearance.

F_5W

The F_4 spikes are planted as spike-to-row 0.5 m in Terbol station under irrigation, and artificial inoculation with yellow rust is imposed. Individual rows are then selected on the basis of their appearance under high-input conditions.

Stage 1 W ($F_{5:6}W$)

The F_5 rows are planted as row-to-plot of 3 m in length at Kfardan station using an augmented design. The yield data are used to select the best 24 entries each year and two spikes each are harvested and threshed individually. The seeds and spikes of the selected entries are stored until further use.

Pre-INW (F_7W)

To provide pure seeds to partners, the two spikes for each of the selected entries for Stage 2 are planted as spike-to-row in Terbol under irrigation, each row is visually selected, and one row per entry is harvested and put in storage.

Stage 2 W ($F_{5:7}W$)

Every 5 years, the best 24 entries from all Stage 1 W tests conducted are retrieved from storage and used to run a joint Stage 2 W trial at two stations (Kfardan and Annoceur). The Stage 2 trial uses an alpha lattice design with two replicas and a plot size of 3 m. The yield data are used to select the best 24 entries at each station.

INW ($F_{7:8}W$)

The seeds obtained from the F_7 spike-to-row in Terbol are retrieved from storage and planted in AREC in 7 m long plots for multiplication. Only the 48 entries selected for Stage 3 testing will be used.

Stage 3 W ($F_{5:8}W$)

The 48 entries selected from Stage 2 W are tested a second time at two stations (Kfardan and Annoceur) in an alpha lattice design with two replicas, and on a plot size of 3 m. The yield data are used to select the best 12 entries at each station, for a total of 24.

Stage 4 W IFWDON

The 24 entries selected from Stage 3 W are then provided to partners around the world based on their requests at https:/indms.icarda.org/
On average, 30-40 requests are received each year. These entries are distributed as two reps alpha lattice of 5 m in length and using 1,800 seeds per plot.

Recycling of IFWDON as a crossing block

Each 5 years, the 24 entries of IFWDON are recombined to design 100 new crosses for the facultative winter program. In addition, the top four entries identified by partners are incorporated in the spring crossing block to deliver novel and useful alleles.

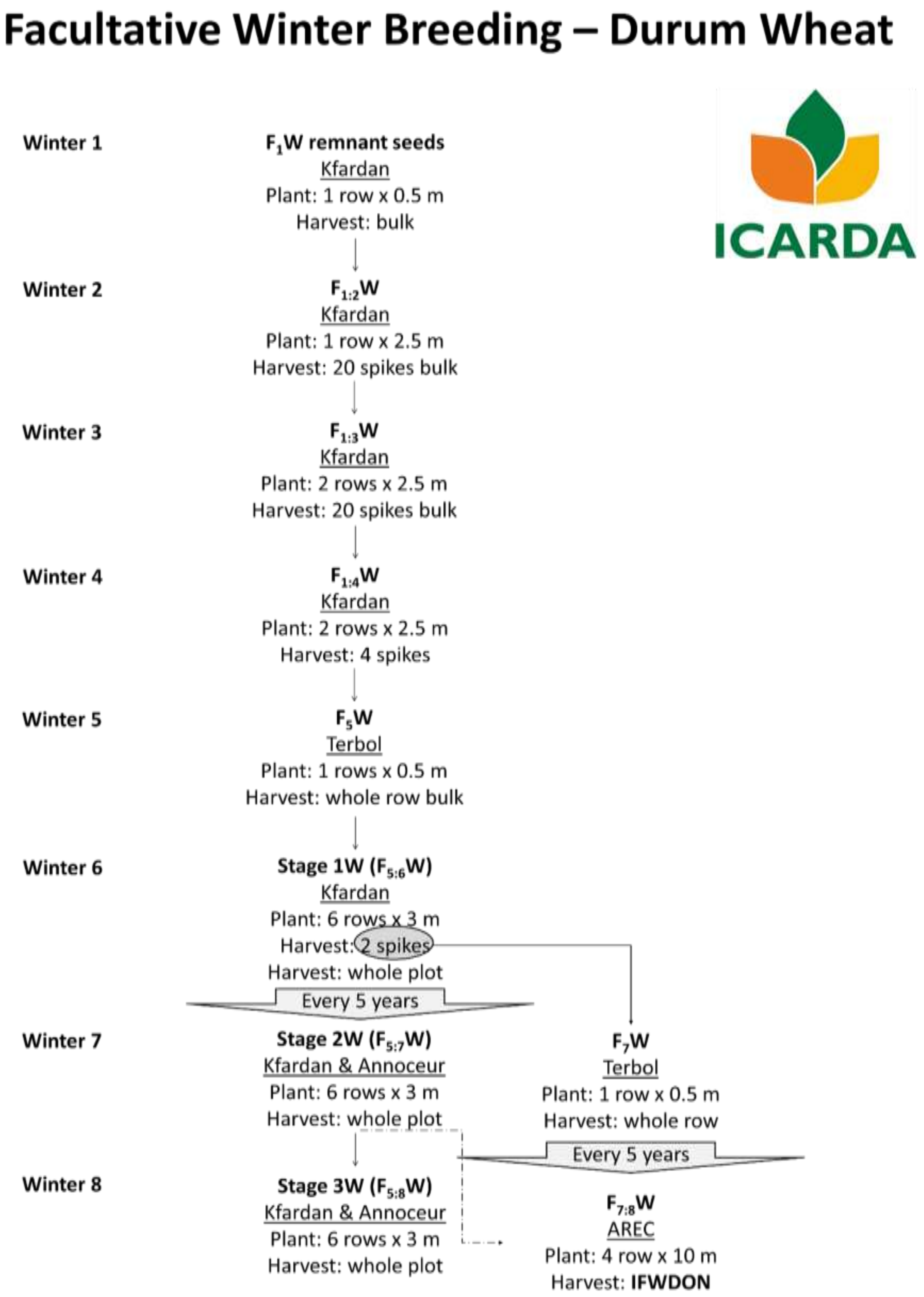


Figure 42. Schematic representation of ICARDA's facultative winter program

Pre-breeding and discovery activities

At ICARDA, the concept of pre-breeding is used to define three key aspects:

- Creation of new knowledge, which entitles the identification of good phenotyping or molecular methods to select for targeted traits.
- Allele discovery through the identification of good allele sources to respond to the trait requirements for specific PPs.
- Forward breeding by identifying future-needed traits to adapt the germplasm to the changing climates.

The first two points are addressed based on specific PP needs (see list of key traits). The third point is addressed on the basis of the 2017 Arab Climate Change Assessment Report, which represents, to date, the most accurate prediction for the next 50 years within the region targeted by ICARDA's breeding effort (Fig 43). In this report, it is indicated that the temperatures in the region will increase by +1.9 ^{o}C by 2050, and by +4.8 ^{o}C by 2100. This, in turn, will result in an average reduction of -100 mm rainfall by 2100, a doubling of days of >40 ^{o}C, and a general decrease in the availability of underground water for irrigation. In other words, it can be expected that there will be more initial and terminal droughts associated with an increase in the frequency and length of heat waves; shorter growing seasons; reduction of the irrigated surface/amount of water provided; and potential for the development of currently unseasonal pests and diseases.

Most of these aspects are already targeted in nearly all PPs, so there is a good alignment between current breeding efforts and forward thinking. The only element that requires more attention in advance is the work on pests and pathogens. The Warrior race of yellow rust is a good example of a fungal disease that was historically limited to wet and cool years, which has now adapted to warm and dry conditions. Similar evolutions can be witnessed for powdery mildew and fusarium head blight, making it critical to observe the evolution of these pathogens' to be prepared to address them.

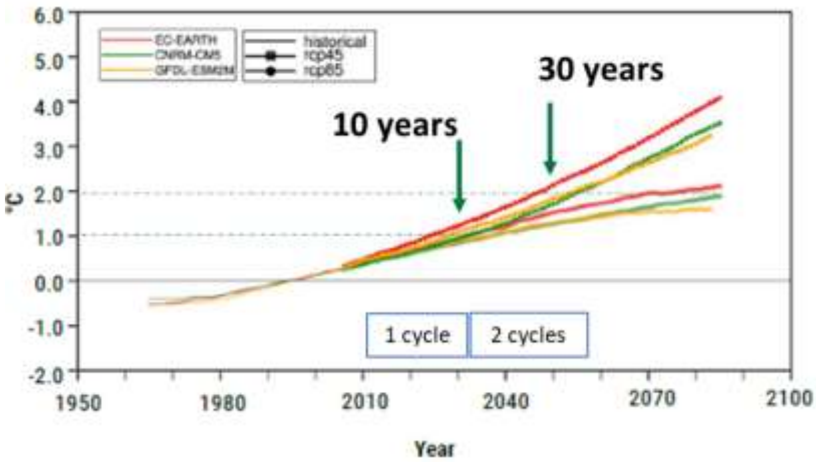


Figure 43. Climate change models for the Arab region. Adapted from Arab Climate Change Assessment Report, 2017

Identification of key traits for pre-breeding efforts

Based on the requested PPs and their prioritization, the following traits – and their priority to focus on pre-breeding efforts – have been identified:

Phenology

The spring-type PPs require flowering time that is super-early, early, and medium. These traits are readily available within ICARDA's germplasm, with most of the Stage 2 lines being early, the Ouassara-derivatives super-early, and the lines obtained from crosses with landraces, European germplasm, 'Miki-3'and CIMMYT elites being medium. Spring phenology definitions can be easily obtained by comparing the days to heading at Marchouch, Terbol, and during the summer off-seasons.

The winter-type PPs require flowering time that is early, and medium. These traits are incorporated in ICARDA's facultative winter program, with most of the germplasm being early or medium. Winter phenology definitions can be easily obtained by comparing the days to heading at Annoceur, Kferdan, and during the summer off-seasons.

Special attention should be given to the development of 'true winter' durum. This is a unique type of germplasm that is currently not requested by NARS partners, so it should not receive large investment. Yet, there is a theory that anticipating the planting by 2 months might be a strategic way to overcome some of the climate change issues of initial and terminal droughts. Such an approach would require 'true winter' types to avoid flowering too early in winter.

In addition, winters in Central Asia might become milder and, hence, there is potential to adapt durum to fall planting instead of spring as is now the case. In this sense, a collaboration with Prof. Brian Steffenson from the University of Minnesota has been initiated by exploiting four durum landraces he has identified as tolerating the -40° C winters of Minnesota. After testing in Terbol, only one entry resulted as being true durum wheat; it was acceptably resistant to yellow rust and adapted for the task: PI376509 from Romania. Crosses to this landrace were initiated in 2019 and will be used for the facultative winter program. Winter hardiness will be tested at IFWDON level by sharing the germplasm with Iran, Minnesota (USA) and Turkey.

Lodging tolerance

Farmers and breeders in Ethiopia, North Africa and West Asia have a marked preference for taller varieties better suited to providing good biomass as straw. To achieve good height without losing grain yield (harvest index), it is critical to ensure good lodging tolerance. This trait is widely spread among ICARDA's germplasm and even entries above 120 cm in height tend to be standing. The source of this trait is probably 'Haurani' – a Syrian landrace that was widely used through its most successful cross Om Rabi. Still, the best methods to confirm maintenance of this trait is to test Stage 2 entries to conditions that expose yields above 6 t ha^{-1} to strong winds and to soils that do not favor standability. Such methods are used at all stations of Allal Tazi, where disease resistance is normally scored, and Melk Zehr, where fertigation on sandy soils is used. Hence, the use of high seeding rates at these sites during the Stage 2 trial ensures maintenance of this trait in the germplasm. There does not seem to be the need to conduct pre-breeding activities around height and lodging resistance. The continuous integration of landraces into the crossing program, matched with selection against lodging types seems to be enough to ensure good, tall and standing elites, without the need of a targeted pre-breeding effort.

Biotic resistances

Breeders and farmers do not always align on the need for incorporating genetic resistance for specific abiotic diseases. In fact, breeders tend to be very careful in only advancing germplasm that has proven resistant to the main occurring diseases and pests, while farmers often tend to prefer the use of fungicide if the genetic resistance comes at the cost of lower yields. In that regard, it remains critical to ensure high yields before adding genetic resistance to certain diseases. The rusts remain the most critical biotic constraints across the target region of the PP of ICARDA (Fig 44), but several other diseases and pests have prominent roles and are discussed below.

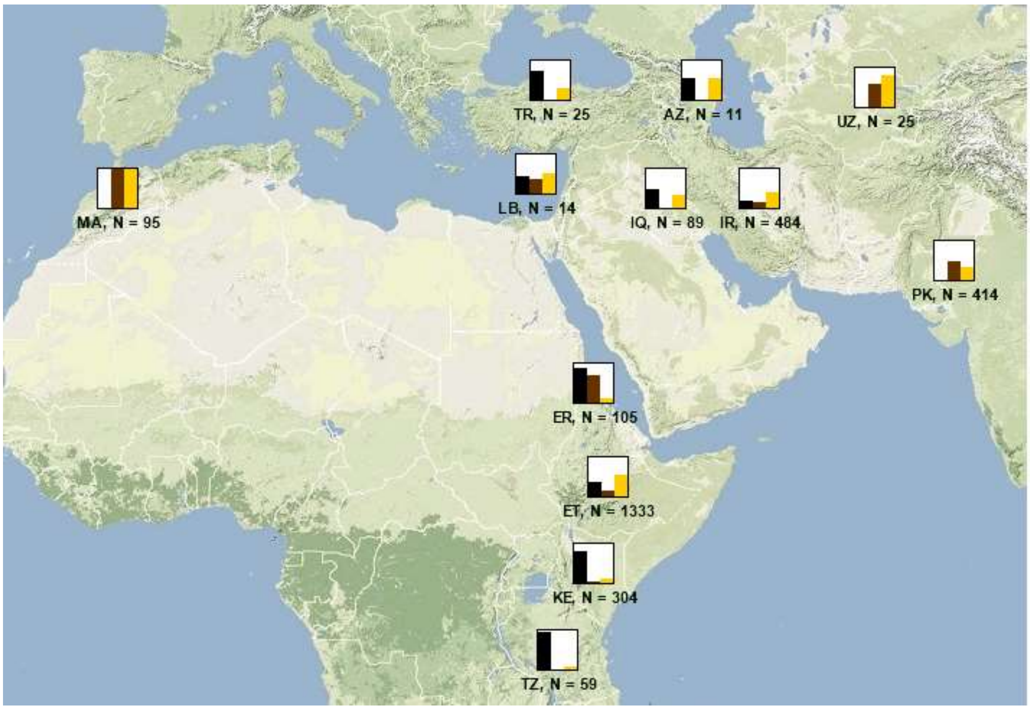


Figure 44. Occurrence of different rust types as obtained from the rust-tracker for the 2016 season

Color code: Yellow= yellow rust, Brown= leaf rust, Black= stem rust.

Leaf rust is historically the most critical fungal disease occurring on durum wheat. For a long time, the 7BL gene Lr14a derived from the very successful cultivar 'Creso' has provided good levels of resistance to this disease but, unfortunately, this resistance broke down in Europe and North Africa in early 2000. In Mexico, between 2001 and 2008, Lr72, Lr27, and Lr31 also lost their resistance to virulent pathotypes. Since this time, many other leaf rust-resistant genes have been described and deployed in durum wheat and the list is now vast, with several of the genes still effective against the pathogen. The Allal Tazi stations present virulent leaf rust races that can be exploited for the selection of good germplasm, with the following pathotypes being reported: BBBQJ, MCDSJ, MCDSS, MCDSD, PBDQJ, and PCDQP, showing a combined virulence on LrB, Lr1, Lr2c, Lr3, Lr3bg, Lr10, Lr14ab, Lr17, Lr20, Lr23, Lr26, Lr28, and Lr39.

For ICARDA's germplasm, the most interesting sources of resistance have been Tunsyr and Geromtel, linked to the KASP markers usw222, usw215, and usw218, which are present in 70-80% of the Stage 2 germplasm (Table 17) (Kthiri et al., 2018). In addition, Lr19/SR25/YP (CIMwMAS0135) is a widely resistant major gene, which has been only partially exploited in durum breeding because of the yield Drag it typically causes. The team of Prof. Ceoloni at the University of Viterbo in Italy has been able to shrink the Lr19 fragment to remove the linkage Drag and the following line has been incorporated in the crossing program since 2016: Simeto.Lr19[R5A83(R5210HOM+)]. However, this entry has proved hard to use, as most germplasm deriving from it had delayed flowering.

With some efforts, several good-yielding lines were obtained in 2019, such as IDON44-12 Ouassara1/SimetoR5210+, and IDON43-05 Ouassara1/SimetoR5210+. An additional source has been the line 'Sebatel', which was proven resistant to all three rusts, but the definition of the resistance genes it carries is still ongoing.

Lr34, Lr46, Lr67, Lr68, Lr74, Lr75, Lr77, and Lr78 are Adult Plant Resistance (APR) genes that have been reported in hexaploidy wheat (only Lr46 is present in durum), and could be further exploited for building up resistance.

From a pre-breeding perspective, leaf rust remains the most critical of durum's diseases, so it is worth continuing investment in this direction. However, with the vast number of genes that have been identified and validated (i.e. from Amria, Arnacoris, Byblos, Gaza, Saragolla, and several others) it is best to focus on exploiting the available sources rather than attempt to discover new ones.

Stem rust is also a challenging durum disease, especially after the spread of the Ethiopian-like race TKTTF (Digalu) in North Africa, South Europe and West Asia. This pathotype is virulent on most available Sr genes, except: Sr2 (APR durum), SrAes7t, Sr9e (durum), Sr11 (overcome by TTKSK), Sr13 (durum), Sr14 (durum), Sr17 (durum), Sr22, Sr24, Sr26, Sr27, Sr28 (durum), Sr31 (overcome by TTKSK), and Sr47 (durum). A total of five reported genes confer quantitative APR, but only two are located on the A-B genomes: Sr2 (marker csSr2, Sr2_ger9 3p) and Sr56 (Swiss winter wheat Arina is the donor, STS marker sun320. Sr25, which is coupled with Lr19 and present within the Ceoloni's stock, was reported as being overcome by new TKTTF races in 2017, so it is no longer of interest for breeding stem rust resistance (Patpour et al., 2017).

ICARDA's elite line 'Sebatel' was reported as harboring Sr2 (marker csSr2, Sr2_ger9 3p), Sr13 (Kronos: cloned gene, marker Sr13F/R), and Sr22 (STS marker cssu22). The introgression of these genes from 'Sebatel' is hence very strategic to obtain good, stable resistance (Haile et al., 2012). In addition, 'Waha' = 'Cham1' was confirmed to harbor Sr35, a cloned gene. Prof. Steven Xue of USDA-ARS Fargo has provided us with the following stocks: RWG35[Rusty/3/Rusty5D(5B)/DAS15/4715D(5B)], RWG38[Rusty/3/Rusty5D(5B)/DAS15/4715D(5B)], which incorporate Sr47 and SrAes7t, respectively. These are precious genes for deployment, but their background is the poor-performing durum rust-susceptible check 'Rusty', so a targeted introgression effort must be carried on to ensure the transfer of only the targeted loci.

The TKTTF race occurs during the summer off-season in Terbol and Annoceur, and with low frequency during the winter season in Allal Tazi and Kfardan. Hence, good selection against it can be made by segregating populations. For pre-breeding, there is a strong need to validate/convert the main Sr complex of 'Sebatel'/Cham 1' into easy-to-use KASP. These should be merged with the stocks available from Professor Xue. There does not seem to be a wider scope to seek new loci for resistance since several are readily available from the literature with their associated marker.

Yellow rust was, for a long time, a major challenge for durum wheat cultivation but, during the last decade, the work conducted – and the race changes – have ensured a gradual disappearance of virulence on durum wheat. In 2019, for the first time in a long time, several of ICARDA's F_4 populations had to be discarded because of yellow rust virulence in Terbol. The pathotype responsible for this virulence has been defined as Yr10, Yr24 virulent. The most common races of yellow rust present in North Africa and West Asia are: PstS2v3, PstS2v27, Warrior, PstS11, PstS13, and PstS14. These combine virulence on Yr genes: 1, 2, 3, 4, 6, 7, 8, 9, 17, 25, 27, 32, AvS. Hence, it is possible that the spread of the new race to Lebanon has knocked off Yr10, a resistant gene present in durum wheat. Still, the results from Izmir and Terbol confirmed good levels of resistance in the Stage 2 and Stage 3 germplasm. Therefore, introgression of novel yellow rust-resistant genes does not appear as a current priority, but evolution of the pathogen virulence needs to be carefully monitored. In addition, there is value in conducting targeted seedling and adult plant screening to describe the current set of resistant genes present in the germplasm.

Septoria tritici blotch (*Zymoseptoria tritici*) is an expanding disease in the coastal high-rainfall areas of North Africa and West Asia. It typically occurs when rainfall exceeding 450 mm is combined with extended cool periods; however, tough new races better adapted to lower moisture and higher temperatures have started to appear. This pathogen is of particular interest for those PPs targeting higher rainfall areas. Due to the specific aim of ICARDA's breeding activities to serve Drylands, this disease has received somewhat less attention. Nevertheless, annual scoring of Stage 3 and Stage 4 trials for *Septoria tritici blotch*-resistance is conducted at the CRP WHEAT platform of Beja, Tunisia, and scoring of Stage 2 under artificial inoculation in Guich. The results indicate that the germplasm segregates for this pathogen, with 1/10 of the entries showing adequate levels of resistance.

The Ouassara, Hessept, Chicca, Canzone, SWAlgia, and Helab lineages appear to carry resistance loci. So far, 21 genes have been identified to confer resistance to *Septoria tritici blotch* and were designated Stb1 to Stb18, StbSm3, StbWW, and TmStb1, but their distribution in ICARDA's germplasm is as yet unknown.

For pre-breeding activities, it would be important to determine which resistant loci are available within ICARDA's germplasm. The investment for building resistance to this disease should otherwise be limited, and only sought as an additional trait.

Fusarium head blight (*Fusarium gramineaurum*) is an extremely challenging disease that is ravaging the harvests in many northern regions. It typically occurs when rains fall after flowering, in combination with medium and hot temperatures. This pathogen is not listed in any of the PPs targeted by ICARDA, so no substantial attention has been dedicated to it. However, there is the risk that the fungus will expand to the Southern coast of the Mediterranean, so it might be interesting to monitor the availability of potential sources of resistance. To date, the Sumai 3 source has been the most widely exploited to obtain a Type II resistance. Unfortunately, this locus is not entirely effective in durum wheat, and mixed results have been obtained thus far. The teams in Northern Europe, Canada, and North Dakota (USA) are among the most active in describing novel sources of fusarium head blight resistance for durum wheat. One West-Asian landrace 'Shahba' has been described by fusarium head blight testing in Canada, Italy and Uruguay as providing moderate levels of resistance (Table 21). This landrace was originally provided by ICARDA and has been used for crossing since 2017.

Table 21. Multi-location testing of ICARDA's germplasm against fusarium head blight

		Brandon (4 reps)						Indian Head (2 reps)						Uruguay platform (1 rep)			
Name	Source	Incidence*		Severity***		Index***		Incidence***		Severity***		Index***		Incidence	Severity	Index	DON
SHABHA	DCC-809	90.0	d	36.3	i	34.0	i	65.0	cdefghi	40.0	ghijk	26.0	jklmnop	0.1	20.0	0.0	3.7
Strongfield	Strongfield	95.0	abcd	40.8	i	39.4	i	42.5	hij	32.5	jk	14.3	nop				
AWALI-1	DCC-422	93.8	bcd	47.0	i	45.4	i	22.5	j	27.5	k	6.4	p	10.0	60.0	6.0	11.4
Icarnada	IDyT37-18	96.3	abc	71.3	fgh	69.4	fgh	37.5	ij	27.5	k	10.0	op	70.0	50.0	35.0	
Louiza	AfN-002	93.8	bcd	70.0	gh	66.6	gh	50.0	fghij	35.0	ijk	22.0	klmnop	60.0	40.0	24.0	
Younes/TdicoAlp	AfN-009	95.0	abcd	66.3	h	63.5	h	70.0	abcdefgh	47.5	efghijk	28.8	hijklmnop	10.0	30.0	3.0	23.9
Faraj	AfN-006	93.8	bcd	71.3	fgh	68.8	fgh	90.0	abcd	55.0	cdefghij	49.5	cdefghijk	20.0	20.0	4.0	
Icajihan2013	IDyT37-06	92.5	cd	77.5	efgh	73.0	efgh	87.5	abcd	52.5	defghij	46.4	defghijklm	60.0	80.0	48.0	
Mrb5/TdicoAlpC	AfN-004	98.8	ab	80.0	defg	78.9	cdefg	67.5	bcdefghi	52.5	defghij	41.1	fghijklmn	70.0	30.0	21.0	
Icarhani	IDyT37-10	97.5	abc	80.0	defg	78.6	defg	72.5	abcdefgh	52.5	defghij	45.6	defghijklm	60.0	70.0	42.0	
Miki3	IDyT37-20	97.5	abc	87.5	abcde	85.5	abcde	52.5	efghij	35.0	ijk	18.4	mnop	80.0	40.0	32.0	
OMRRUF-2	DCC-509	98.8	ab	95.0	ab	93.9	ab	60.0	defghi	32.5	jk	18.9	lmnop	30.0	70.0	21.0	
Secondrue	AfN-016	100.0	a	96.3	ab	96.3	a	47.5	ghij	35.0	ijk	20.0	lmnop	70.0	60.0	42.0	
Icakassem1	AfN-013	100.0	a	97.5	a	97.5	a	65.0	cdefghi	45.0	fghijk	27.0	ijklmnop	80.0	80.0	64.0	
Waha	AfN-011	100.0	a	92.5	abc	92.5	abc	95.0	abc	37.5	hijk	35.5	ghijklmno	80.0	70.0	56.0	
	CV:	3.756		10.16		11.35		19.4		20.56		27.98					
	LSD:	5.161		12.322		13.613		32.087		23.847		28.203					

Two additional sources of fusarium head blight resistance were provided by Professor Xue: 10LND791[Lebsock/PI277012/Lebsock] and 15PRO343[Sumai3/10Ae564/Lebsock], which incorporate a 5AL and Sumai3 resistance loci, respectively. These are in Lebsock background, an unadapted cultivar. Another fusarium head blight resistant source was provided by Professor Ceoloni as NAU405Yr26/RRR50-10-

50/Simeto/3/F898145/3*Karur=RRR/Karur, which carries resistant sources from *Thynopirum ponticum*. This is in Karur background and is a French cultivar with facultative winter behavior. Currently, there is no phenotyping facility available at ICARDA for fusarium head blight screening.

For pre-breeding activities, it would be best to work with advanced research institutes to combine these three sources of resistance onto ICARDA's elites, and provide this important germplasm to international partners for their use.

Hessian fly is a nasty pest common to North Africa and West Asia. Its damage can be exceedingly widespread when planting is delayed to the second half of December. This is often the case for those farmers that do not have access to their own agricultural implements and are therefore obliged to wait in order to rent them. In addition, heavy rainfall in October can delay soil preparation and planting operations, or delayed rainfall in December can cause late germination.

ICARDA, INRA-Morocco, and Kansas State University have worked together since 1990 to deliver Hessian fly-resistant varieties to Moroccan farmers. In 2007, this work resulted in the registration of the variety 'Faraj', which carries a short segment from *Triticum araraticum*. In 2019, the use of molecular tools allowed this locus to be mapped to the short arm of chromosome 6BS and to tag it with the KASP marker BS00072387 (Bassi et al., 2019). The positive allele of this locus is currently present in 9% of the germplasm and requires further upscaling. The same study also enabled the identification of a second exploitable locus (QH.icd-2A) in the line Younes/T.dicoAlpCol//Korifla, and H31 in several entries. Combination of these three sources seem enough to ensure resistance to the pest. Screening for the pest is also readily available in seedlings in the growth chamber at Guich, or in field at the station of Sidi el Aydi when delayed planting is used.

For pre-breeding activities, the three sources of resistance need to be a pyramid. In addition, it would be important to tag the remaining two sources with molecular markers to facilitate their MAS. Stage 2 and Stage 3 testing for Hessian fly will be continued.

Crown or root rot (*Fusarium culmorum* and *pseudograminearum*) is one of the major diseases of Dryland agriculture. It occurs almost inevitably when Dry conditions favor its infection at the end of the year, and causes severe yield losses. This disease is a major target of ICARDA's breeding program. Empirical selection for resistance has been conducted over the years in Tel Hadya, Sidi el Aydi, and Marchouch stations. However, true selection has been thus far hindered by the difficulty of generating sufficient homogeneous pressure in the field, since the disease tends to create patches rather than being spread evenly across a trial.

The team at the University of Adelaide (Prof. Woolworth and Prof. Able) has been the only one capable of identifying truly superior durum germplasm, and is now working to deliver it into varieties. The parental line SSD1479-117 (1206-2/5/6/3*WID902) has been kindly provided by them and its use in the crossing program was started in 2019.

In parallel, the team of Prof. Bentata at INRA has also been trained to screen for this pathogen in glasshouse conditions and their tests with multiple biotypes have confirmed that the ICARDA line Icaverve and Berghisyr, and the Moroccan cultivars Marouane and Faraj also present good levels of resistance (Table 22). In addition, the work using speed breeding facilities by Alhamad et al. (2019) confirmed that ICARDA's line Outrob (=Fadda98) harbors a QTL on chromosome 6B that allows for reduced infection of *Fusarium pseudograminearum* to levels similar to the bread wheat resistant cultivar Sunguard.

The team of Prof. Ceoloni has also reported the transfer to durum wheat of a strong QTL for *Fusarium culmorum* resistance (Fhb-7EL) from *Thinopyrum elongatum*. The line R69-9/R5 carries both the Lr19 segment described previously, and this is useful QTL for root rot resistance. Several crosses have been developed at the University of Viterbo by this team to integrate these resistances into ICARDA's elites Korifla and Secondrue: V15x49, V15x39, V15x44, V15x86, V15x42, V15x82. These crosses were integrated in the 2018-19 season and the first round of field selection was completed.

It is therefore important to target crosses that allow introgression of all four resistant sources. In addition, it is critical to increase the investment in root rot screening of all Stage 2 entries using glasshouse conditions. Finally, targeted molecular studies should be conducted to identify the loci harboring these resistances and convert them to easy-to-use KASP markers for MAS.

Table 22. Response of different germplasm to 10 Moroccan *Fusarium culmorum* biotypes

Name	Origin	Isolate 4	Isolate 3	Isolate 7	Isolate 5	Isolate 6	Isolate 1	Isolate 2	Isolate 9	Isolate 8	Isolate 10
		Severity of disease (crown and stem browning indexes)									
Marouane	INRA	MR	MR	MS	R	MR	R	R	S	MS	S
SSD1479-117	U. of Adelaide	MR	R	MR	MR	MR	MR	MS	MS	S	S
Icaverve	ICARDA	S	R	S	MS	MR	MS	MR	MS	S	MR
Berghisyr	ICARDA	S	MR	MR	S	R	MS	MR	S	MS	S
Berghisyr2	ICARDA	S	S	MR	S	R	MR	S	S	MS	MR
Icaverve2	ICARDA	MS	R	S	MS	MR	S	S	S	S	S
Faraj	ICARDA/INRA	S	S	S	MS	MS	MR	MR	MS	MR	MR
Marjana	INRA	S	MR	MR	MS	S	S	MR	MS	MR	S
Jabal	ICARDA	S	MR	S	MS	MS	S	MR	S	S	MR
Toumouh	ICARDA/INRA	S	S	S	MR	MS	S	MR	MS	MR	MS
SSD1479-106	U. of Adelaide	MR	MR	MS	MS	S	MR	MS	S	S	S
Hessept2	ICARDA	MR	S	S	MR	MS	S	MS	S	S	MS
Amina	ICARDA	S	MR	S	MS	MS	MR	S	S	MS	S

Hessept1	ICARDA	MR	S	MR	MS	S	S	S	S	S	S
Luiza	CIMMYT/INRA	S	S	S	S	MS	MR	MS	MS	MS	MS
SSD1476-137	U. of Adelaide	S	MR	MS	MS	S	S	MS	S	S	S
SSD1476-233	U. of Adelaide	S	MR	MS	S	S	MS	S	S	S	S
Icambel	ICARDA	S	MR	S	MS	S	S	S	S	S	S
Icambel2	ICARDA	S	S	S	S	S	S	S	S	S	MR
Karim	CIMMYT/INRA	S	S	S	S	S	S	S	S	S	S

Abiotic tolerance

The need to adapt germplasm to changing climates is the main and most important activity conducted by ICARDA’s durum wheat breeding program. Nearly all PPs require tolerance to drought and terminal heat. However, these represent complex traits with many minor gene effects. All stages of the breeding programs are conducted under terminal drought and heat conditions at the Marchouch station, and in both off-seasons. This allows for good selection pressure in favor of adaptation to these two stresses. Still, this does not ensure the introgression or identification of novel allele sources. In this sense, targeted pre-breeding activities are needed to identify and exploit these genes.

Heat tolerance is relatively the easier of the two stresses, since it can be more easily quantified. Escape from terminal heat by deploying early-flowering genotypes is a simple and strategic approach to reduce the damage, but it also results in a general loss of yield potential. The use of delayed planting at the main station can help identify these escaping entries, but it does not necessarily help in identifying novel usable alleles for true tolerance to heat. Two pre-breeding strategies have been adopted instead: the imposition of plastic tunnels at the time of flowering to increase the temperature by +5-10° C, and planting along the Senegal River under continuous >32° C max daily temperatures (Fig 45). Both studies confirmed that heat stress can reduce yields by up to 50%, by heavily affecting the ability of pollen to fertilize the ovary (grain number per spike) and promoting early Drying (reduction in biomass) (Sall et al., 2018a,b & 2019; El Hassouni et al., 2019). The combination of the two screening methods (Fig 45) has identified Faraj, Kunmiki, Berghouata1 and Ourgh as ideal sources for heat tolerance.

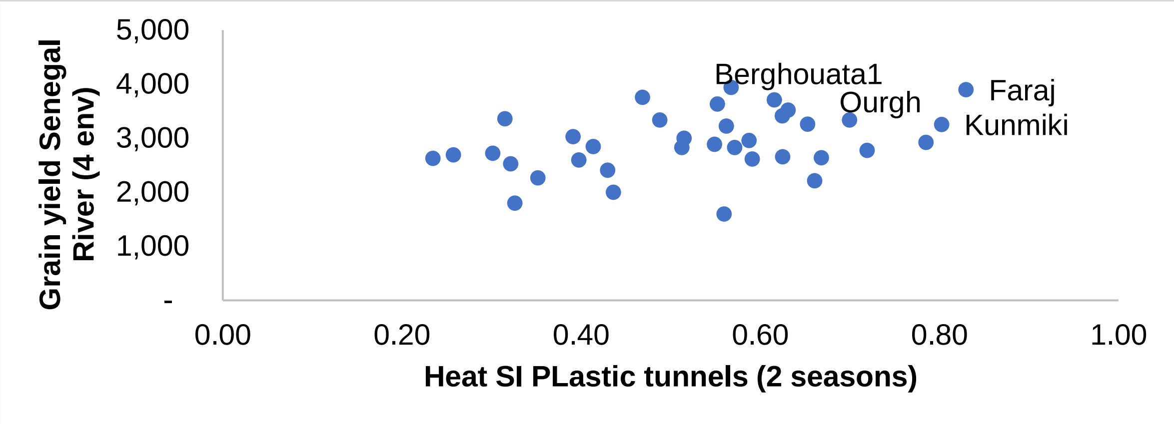


Figure 45. Heat tolerance testing along the Senegal River and in plastic tunnels imposed at the time of flowering (top photos); results of the combined heat stress selection (bottom graph)

Winter hardiness research has been limited due to the narrow scope and limited surface area of true-winter durum. Nevertheless, with changing seasons and unpredictable weather patterns, the occurrence of frost at flowering might take a more prominent role in the near future. Also, the potential to develop true winter hardy durum varieties might help the expansion of the cultivated area of this crop. To date, the screening has been done only at field level using the Kfardan station (Lebanon), in combination with targeted screening at the University of Minnesota and partnership activities with NARS in Iran and Turkey. A durum facultative winter program is carried out at Kfardan for this scope. In addition, Prof. Steffenson provided seven landraces that he tested for three seasons under harsh winter-kill conditions in Minnesota (below -30° C extremes). Additional testing in Terbol, Lebanon has identified PI376509 (Romania) as the most usable for crossing due to its low susceptibility to yellow rust and good lodging resistance (Table 23). The entry PI293422 (Ukraine) was also identified as highly promising, but its extended phenology makes it harder to cross and it seems better targeted for the environment with very long winters, which are not present within ICARDA's mandate area.

Table 23. Screening results of landraces identified as tolerant to winter kill by the University of Minnesota (Prof. Steffenson)

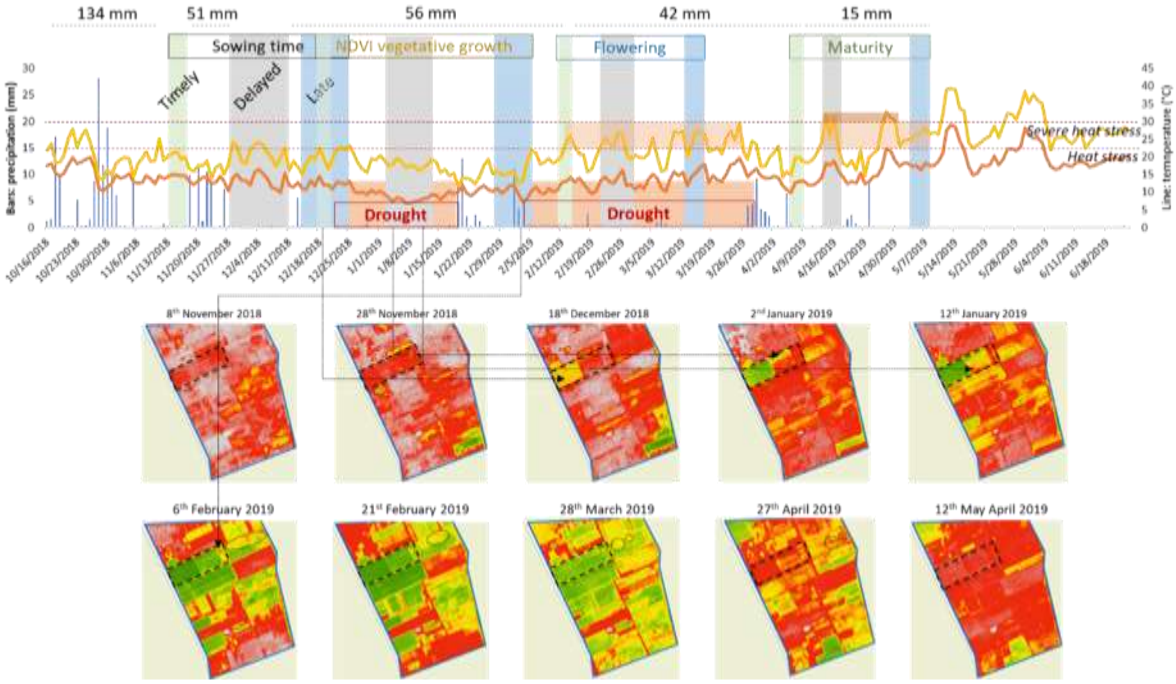
Source	ID	Species	Days to heading	Days to maturity	Plant height	YR	Lodging
PI 341480	Isparta Turkey	subsp. Durum	162	210	147	70MS	
PI293422	Kharkiv Ukraine	subsp. Durum	173	210	135	0	
PI376509	Romania	subsp. Durum	162	210	145	5MR	10%
PI626790	Bakhtiari va Chahar Iran	subsp. Durum	158	210	140	80MS	20%
PI197732	Malmohus Sweden	subsp. Aestivum	180	210	119	0	
PI190156	Kharkiv Ukraine	subsp. Aestivum	160	210	130	10MS	
PI345127	Serbia	subsp. Aestivum	160	210	132	100S	50%

Drought tolerance is the most challenging of the traits and the most complex to screen consistently. However, it is also the most critical aspect of ICARDA's germplasm and the one for which the largest budget has been invested. There are broadly two types of drought: early in the season and terminal. Terminal droughts are the most common and can be defined as the unavailability of moisture and rainfall between the week preceding flowering until harvest (Z39-Z90). This stress results in an overall shortening of the grain-filling period and a reduction in TKW (1,000-kernels weight). Early droughts are fortunately rarer, but also the most damaging. They can be defined as a lack of available moisture occurring between germination and stem elongation (Z10-Z30). In this case, the stress mostly affects the capacity of the plant to produce tillers and biomass, which, in turn, results in severe losses of grain number per surface area and harvest index.

Figure 46 presents an example from the 2018-19 season at Marchouch station, when two drought stresses occurred early and late in the season. The delayed planting was exposed to both stresses, while the timely planting somewhat avoided the early drought.

Screening for both types of drought is complex, but some strategies have been developed. *Field level screening* remains the most scalable approach. Multi-location trials are used to expose the germplasm to contrasting conditions. The stations of Marchouch, Annoceur, Sidi el Aydi and Kfardan provide reliable drought occurrence both early and late in the season. To evaluate the tolerance to early droughts, the number of fertile spikes per m^2, biological yield, harvest index, and number of grains per m^2 are recorded. To evaluate the tolerance to terminal droughts, the TKW is instead used, together with the grain-filling rate calculated as a ratio between TKW and number of days elapsing between flowering and maturity. Further, to account for the GxE effect, the AWAI, Bassi & Sanchez-Garcia, 2017 is used. The combinations of these traits will reveal the most exploitable elite and landrace germplasm to be used in the crossing program.

Figure 47 presents the results across 18 environments, 11 defined as experiencing moisture stress and seven without moisture stress. That study revealed that Isly, ADYT97, Magrour, and Colosseo are the most drought-tolerant elites.
A total of 1,500 landraces were selected via FIGS for different traits. Of these, 96 were field tested at the same 18 environments as the elites. Ten have been selected for integration in the crossing block (Table 24).
To pyramid useful alleles for drought tolerance, the 10 landraces will be top crossed to the four elites.



	Moisture (mm)			Days of heat stress	Grain yield (Kg ha^{-1})	
	Pre-season	Sowing-flowering	Flowering -maturity		Average	Max
Early planting	134	106	42	22	5,080	6,000
Delayed planting	185	59	39	26	3,721	4,615
Late planting	192	55 + 10	50	37	3,100	4,600

Figure 46. Example of occurring droughts during the 2018-19 season at Marchouch, Morocco

Screening under controlled conditions is also important to identify drought-tolerant genotypes. To improve tolerance to terminal droughts, the root system can play a critical role. In regions where the topsoil depth exceeds 70 cm, moisture tends to accumulate in the deepest layers and remain available at the end of the season. Hence, genotypes growing roots that extend to the deepest layers have notable advantages. This is not the case in those conditions where the soil depth does not exceed 40-50 cm. In this case, the effort of growing deeper roots will not result in capturing more moisture, but rather in losing the superficial rainfall before it evaporates.

Figure 48 presents the phenotyping methodology that was utilized to discriminate root types (El Hassouni et al., 2018) and the results obtained for a sub-set of elites. ICARDA's elites IDON37-52, ADYT46 and Bezater demonstrated a deep root system, while Ouassara, Isly and Jabal present a shallow root type. Since this trait is under strong genetic control and its impact on drought tolerance could be demonstrated, it appears as an ideal target for breeding. The variation identified is sufficient for direct exploitation, and crosses should be conducted to pyramid deep root types with the drought-tolerant germplasm identified above.

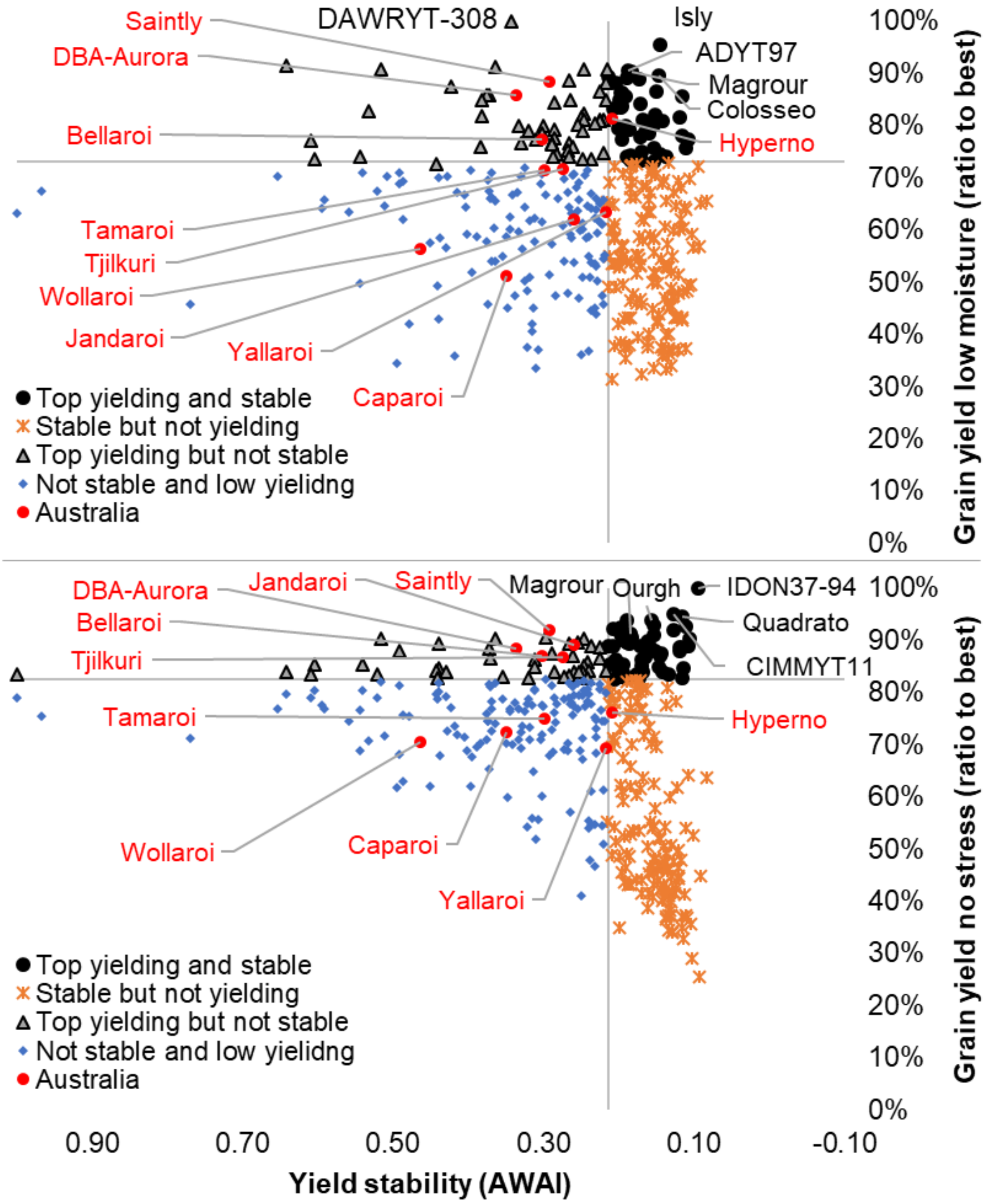


Figure 47. Grain yield vs yield stability (AWAI) for moisture stressed and non-stressed environments

The axis intercepts have been set at the 3rd quartile for both traits. The Australian checks and the top yielding and stable entries are reported in the figure.

Table 24. Landraces integrated in the crossing block after testing 96 selected by FIGS across 18 environments

DurAM Core	Ig	Origin	FIGS Set	Traits	GY drought 15-16	GY drought 17-18	GY moisture 15-16	GY moisture 17-18	Spk.m2 15-16	Spk.m2 17-18	TKW drougth 15-16	TKW drought 17-18	TKW moisture 15-16	TKW moisture 17-18
90	99205	Yem	Dry	GY drought	49%		55%		52%		65%		67%	
171	94615	Tun	Al Kaf	GY drought & moisture	59%		61%		58%		74%		76%	
93	99261	Syr	Dry	GY drought, Spk.m2	44%	69%	60%	60%	50%	81%	71%	81%	75%	95%
83	96186	Jor	Dry	GY drought, Spk.m2	41%	77%	47%	50%	45%	83%	72%	81%	70%	96%
79	85991	Eth	wet	GY drought, TKW	52%		44%		48%		90%		91%	
75	85620	Afg	Dry	GY moisture	43%	65%	50%	73%	55%	66%	70%	77%	74%	99%
89	99022	Tur	Dry	GY moisture	43%	55%	61%	85%	58%	69%	73%	75%	70%	97%
88	98797	Irn	Dry	TKW	40%		49%		49%		88%		92%	
137	90744	Ita	Cold	TKW	35%		59%		44%		82%		91%	
124	85404	Eth	Hot	TKW	47%	52%	57%	45%	53%	69%	89%	93%	84%	99%

Trait values are presented as average to the ratio of the best entry at each site.

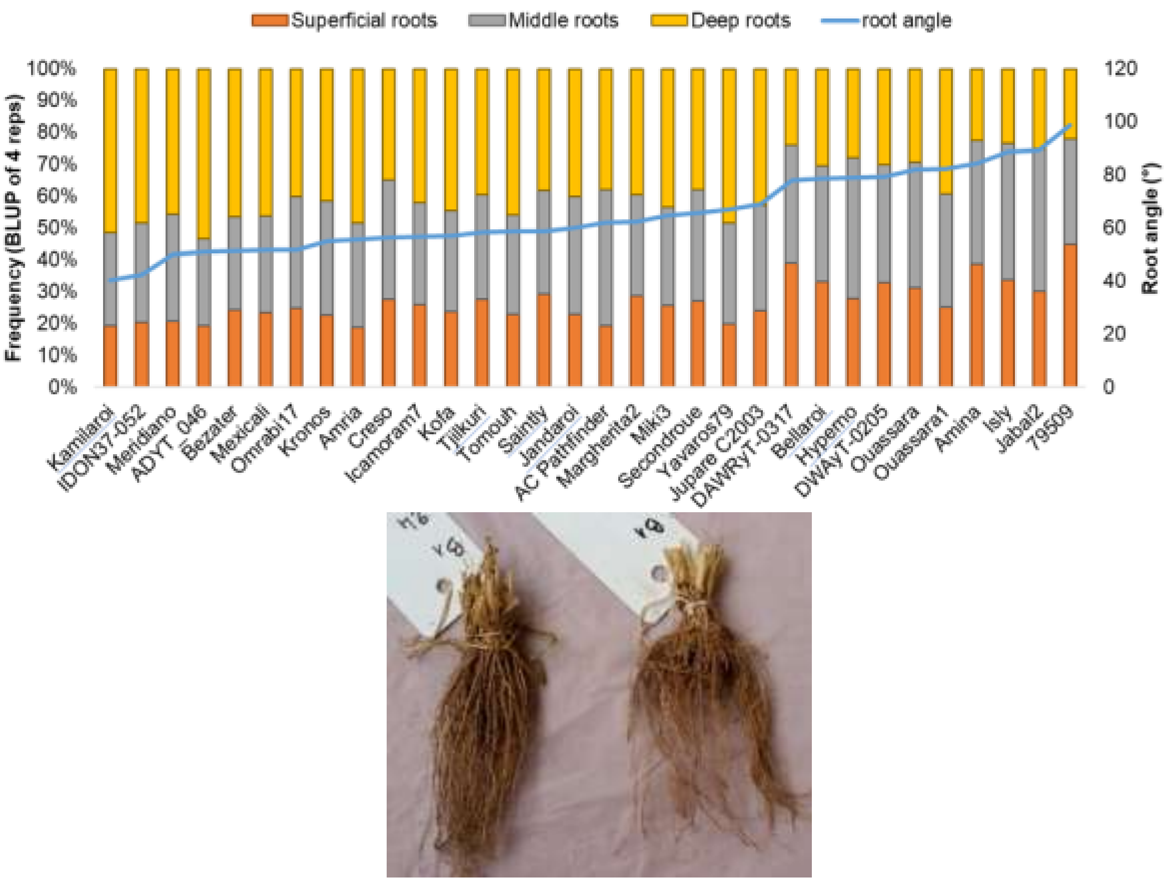


Figure 48. Root types by using pasta strainer method (El Hassouni et al., 2018)

Rheological quality and nutrition awareness

A small portion of farmers in the developing world sell their harvest to the food industry for processing, while the vast majority use their harvests for household consumption or sell it at the local market. There is a long list of traditional durum wheat products (Fig 49) as illustrated in a review by Kezlh et al. (2014) which mentions couscous, flat breads, *freekeh* and pastries. The full list is much longer and includes, for instance, *gofio* from Spain, *defo* and *kinche* from Ethiopia, *pane di Altamura* and *pane carasaou* from Italy, *Dalia* in India, and many more. There are no complete studies yet that have determined the true rheological characteristics that a variety needs to possess to be suitable for these traditional foods. However, farmer surveys suggest that large grains, good yellow color of the seeds (virtuousness), and absence of yellow berry are the most critical characteristics to fetch higher prices at local village markets, and these are also associated by consumers with better end-products. Flavor is also a key characteristic, but its strong subjectivity makes it hard to breed for. Except for the bread types, it appears that SDS is not an important characteristic and hence should not be targeted when considering local food markets.

To date, most of the industrial durum foods from developing countries are produced using imported grains. There are, however, several exceptions such as larger farmers in North Africa, especially in Algeria and Tunisia, some regions of Ethiopia (Oromia), and the area of MP in India, Syria, and Turkey. Also, there is a clear interest by the food industry to start purchasing local grains, but there are no policies in place to favor higher prices for harvests with high-protein content. With this in mind, it is important for ICARDA to integrate this information into the PP agreements, and target top industrial qualities only for those farming systems that will ultimately result in industrial sales. The main industrial products obtained from durum wheat are burghul, cookies, couscous and pasta. Pasta is the most demanding as it requires the most rheological traits: high semolina yield, SDS associated with pasta firmness, high protein content (>13%) and good yellow pigment of the semolina. For couscous and cookies, there is limited interest in SDS and also less emphasis on protein content. For bulgur, the list is also shorter since it is obtained from broken grains without milling into semolina, and hence the only critical trait is TKW. By 2026, the global pasta market is forecast to be worth US$35-40 billion, couscous US$2.2-2.5 billion, and bulgur US$1.3 billion.

Another trend that durum wheat breeders need to follow is consumer demand for more nutritious food. Pasta and couscous are already perceived as highly nutritious foods and the industry is eager to capitalize more on this trend by promoting niche types like whole grains or those made with ancient grains, rich in micronutrients, or derived by mixtures with barley or legumes. In the case of bulgur, there is even stronger consumer interest because it incorporates the bran of the grains, which contain minerals like magnesium, selenium and chromium, dietary fibers, some phenols, and phytates ; and also all the B vitamins except B12. Another super durum food is freekeh, defined by many as the 'new quinoa'. Freekeh is made by burning the wheat spikes at the milk-dough stage. The roasted grains are then cracked and added to soup or salads. Freekeh was apparently discovered by accident when soldiers in the eastern Mediterranean tried to ruin the crop by setting fire to wheat fields. It can be cooked like rice or barley and works well as a stuffing. Wholegrain freekeh is also good for those following a low-GI diet; it contains higher levels of dietary fiber and protein and is a source of calcium, iron, potassium and zinc.

From a breeding perspective, there are some actions that can be sought to align durum with the healthy eating trend, including biofortification to include higher micronutrient and fiber content, use of ancient grains, and good adaptation to organic farming. These characteristics are discussed below.

Figure 49. Examples of traditional durum wheat foods, and those produced at industrial scale
From top left to bottom right: bulgur, *freekeh*, couscous with mixed legumes, pasta from legumes, *kinche*, pana carasao, dalia, and harcha.

Large TKW and semolina yield is the most critical trait for durum wheat, one that was included in all PP agreements to date. It relates to the potential of durum wheat to achieve higher yields, better tolerate terminal droughts, and fetch higher prices at the village markets. It also determines the suitability for several products like bulgur, *freekeh*, and *kinche*, and it has been associated with higher semolina yield. It is a trait with good heritability and should be selected for throughout the breeding cycle.

A comparison of different germplasm sources has confirmed that ICARDA's germplasm achieves the highest TKW (Fig 50). Several GWAS and MP approaches have been conducted for TKW to reveal unique QTLs harbored by ICARDA's germplasm. In particular, Nachit, Gigamor, and Jabal are excellent sources to increase TKW. In fact, the use of CWR in the crossing nearly always results in an increase of TKW (El Haddad et al., 2020). The most suitable exotic germplasm to be incorporated into the ICARDA program is Kronos, which was shown to carry different alleles for increased TKW.

Semolina yield is a critical trait for the industry and several programs have started working on it. However, semolina yield is affected by TKW, moisture, and machineries/strategies used for milling. A simple laboratory mill is not suitable to truly test this character since top extraction rates seldom reach 70%, while industrial mills often exceed 90%. For this reason, it is valuable to measure semolina yield as a selection process in the laboratory, but not as final confirmation. TKW remains a valuable proxy, better suitable for large-scale testing. The use of grain scanners is also valuable to further dissect this trait into grain width and length (Fig 51). While both have good heritability and correlate well with large TKW (r^2 = 0.49 for length and r^2 = 0.58 for width), the width is more influenced by the environment. Hence, it is best to select for long grain genotypes to increase the genetic size of grains, and for grain width to better tolerate droughts. In a recent study of CWR-derived lines, Jabal had the highest grain length, while Sahi was a source for TKW. Combining these two entries would further increase the TKW.

Increased TKW remains a main priority of the breeding program, so pre-breeding investments into identifying novel sources to further increase TKW remain of great interest, especially those coming from primitives and wild relatives such as emmer and pollonicum.

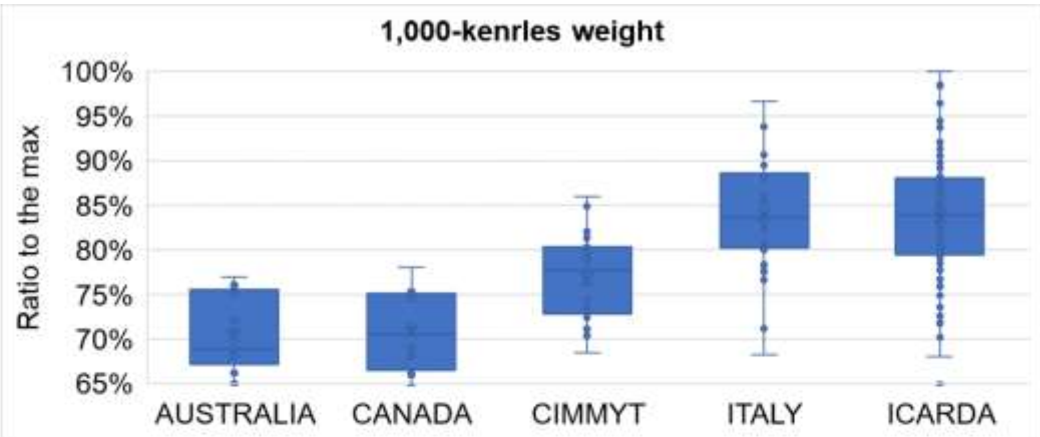


Figure 50. Comparison of germplasm types for TKW combining six environments

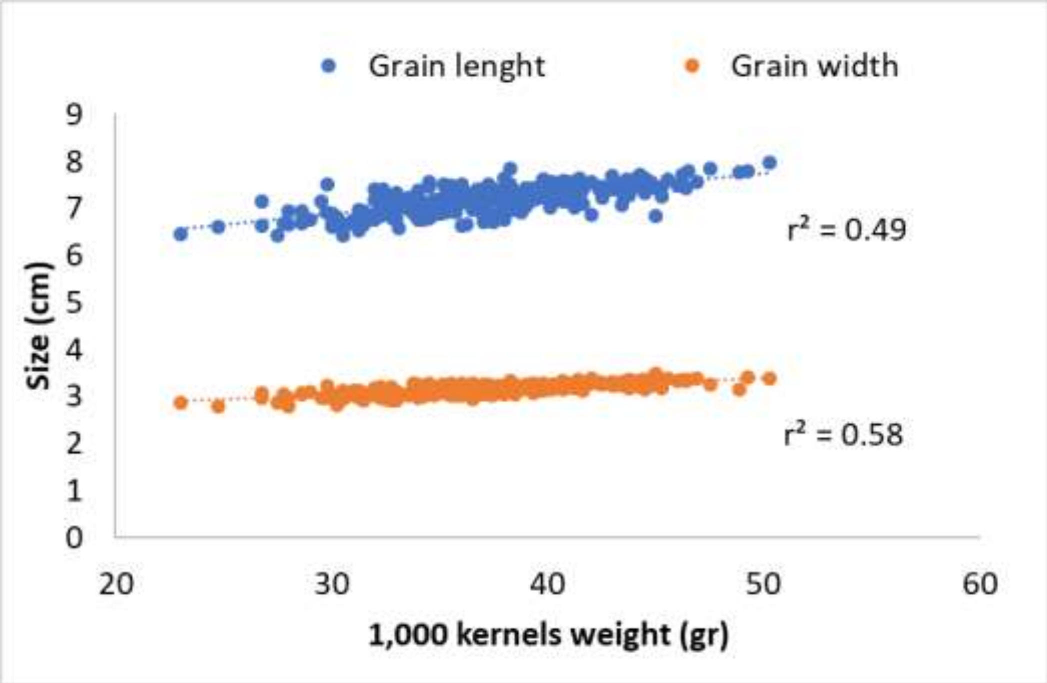
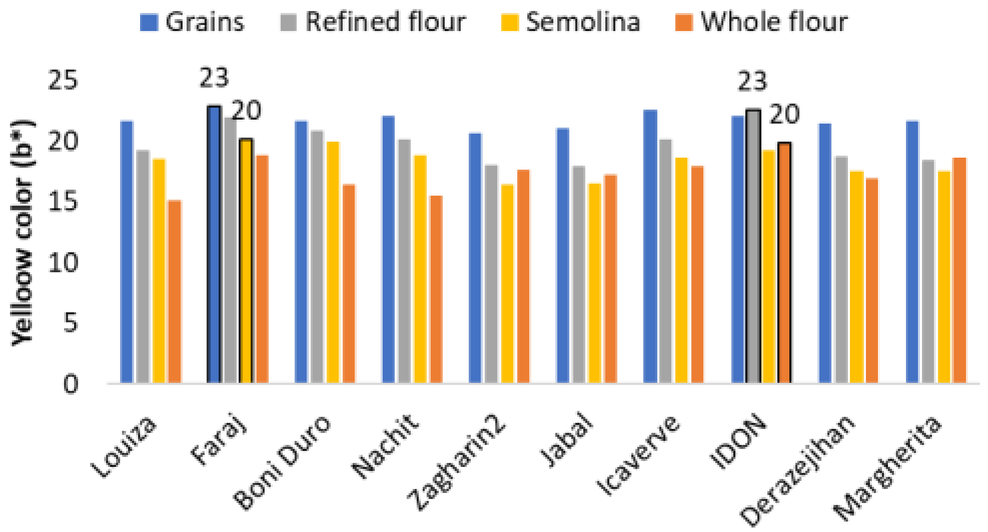


Figure 51. Comparison of TKW characteristics recorded in Marchouch Stage 2 yield trials in the 2018-19 season

Seed color and semolina color are both highly heritable traits controlled by a few genes, but they are not the same. The pasta industry is highly interested in semolina color, while traditional village markets require grains with a stronger yellow pigment. Figure 52 presents the yellow color indicated as b* for the grains, whole flour, refined flour, and semolina. Note how the scores are correlated between grains and refined mill products, but not for whole flour. Still, Faraj and IDON combine top colors for two products. It is important to mention that industrial mills in Africa tend to correct their semolina color via chemical additions.

A comparison between four potential cultivars grown in Morocco under irrigation and one imported batch of seeds from Canada (Table 25) revealed several differences. For instance, testing of the industrial grade semolina *finot,* commercialized by the mill and obtained from imported Canadian grains, reached a b* score of 31.5, but when it was milled in our laboratory, the score was only 24.3, confirming that additives were used by the mill to improve the semolina color. The test also confirmed that good candidate varieties like Sahi have good yellow pigment combined with excellent grain colors. Hence, ICARDA's germplasm is a good source to improve the yellow color of the grains.

For semolina color, Australian cultivars such as Tamaroi and DBA Aurora proved to be the most suitable donors (Table 26). Some markers are also available to follow the transfer of key Australian loci involved in the control of yellow pigment (Table 17): Lpx-A3, Psy1-A1l, and Lpx-B1.1b. Furthermore, the Italian pasta industry, the first to request a stronger yellow color, has reduced its requirements in the past 3 years. The pasta products that are now lining the shelves have a marked brown/yellow color, which was determined to promote an ‘organic perception’ among consumers. As such, it is predicted that the requirements for yellow color will diminish in the next decade, replaced by a stronger need for a brown ‘wholemeal’ color. In that sense, there does not seem to be a need to promote pre-breeding activities for this trait.



Correlations	Whole flour	Refined flour	Semolina
Grains	17%	69%	79%
Whole flour		39%	4%
Refined flour			88%

Figure 52. Comparison of yellow color of different cultivars for grains, whole and refined milling products, and correlation table

Table 25. Quality scoring of four candidate cultivars grown in Morocco against Canadian grains imported by the industry

		Protein content			Yellow pigment (b*)			Mixo
Name	**TKW**	Grains	Flour	Semolina	Grains	Flour	Semolina	**score**
Icaverve	47	14.5	12.6	12.6	20.2	21.2	20.4	7.0
Jabal	48	13.9	12.0	12.9	20.4	19.0	17.7	7.5
Zeina	45	14.7	12.8	13.2	21.1	20.6	21.5	5.0
Sahi	45	15.0	12.6	13.0	21.3	19.6	21.9	5.5
Canada import	39	14.9	13.3	12.8	17.6	22.1	24.3	5.5

Table 26. Quality scores of Australian cultivars averaged across five environments and expressed as ratio to the best entry

ID	SDS	TKW	Yellow pigment
Yawa	94%	68%	89%
Jandaroi	85%	77%	91%
Tamaroi	78%	61%	94%
DBA Aurora	90%	66%	93%

Tjilkuri	76%	69%	92%
Hyperno	90%	75%	89%
Saintly	77%	68%	87%

Low yellow berry and grain protein content are two different traits that are presented together because similar approaches are used to address both. Yellow berry refers to the non-vitreous nature of the wheat kernel. It is not due to the attack of a pathogen, contrary to what many farmers believe, but to the diffraction of light by minute air spaces and discontinuities in the endosperm. This physical discontinuity is highly correlated to protein content and nitrogen availability as reported by several authors (Fig 53). The negative correlation between grain protein content and grain yield is well established (Fig 54). Hence, while the genetic enhancement of protein content and susceptibility to yellow berry have been shown, this typically was achieved with a loss of yield.

In developing countries, a higher protein content of the grains does not result in the farmer being paid extra, with few exceptions, and as such, it does not appear as a major target for ICARDA breeding. Virtuousness on the other hand is of importance since it determines the local price of purchase at the village market, and should therefore be addressed. In both cases, the agronomic approach has been shown to be much more reliable to ensure good protein harvests. In particular, the use of split nitrogen applications with one amount provided pre-sowing (40-80 kg of N ha^{-1}) and one at the booting stage (30-60 kg of N ha^{-1}) has been shown to be extremely effective to control yellow berry, while promoting good yields. Where protein content is a sought-after trait, a liquid application of urea (5-10 kg of N ha^{-1}) just after flowering has the potential to increase protein content by 0.5-1.0%, but without improving yield. Hence, no investment in pre-breeding will be done for these traits, but rather management strategies will be preferred.

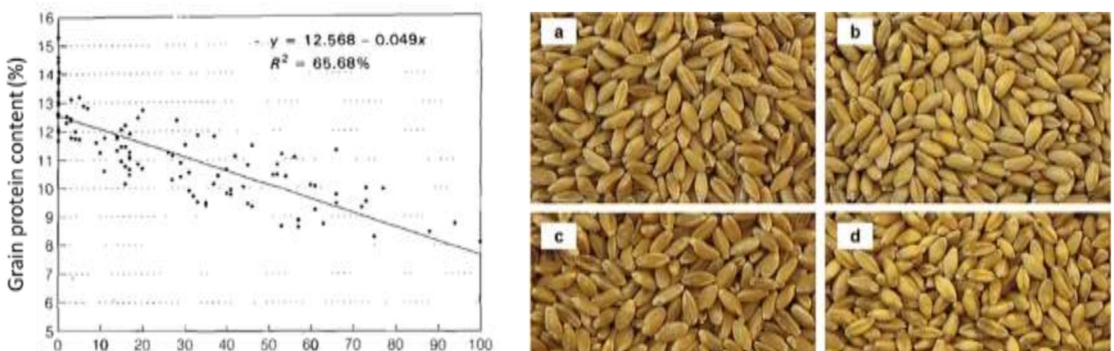


Figure 53. Relationship between protein content and yellow berry (Raath et al., 2013) and a representation of different levels of yellow berry (Xia Fu et al., 2017)

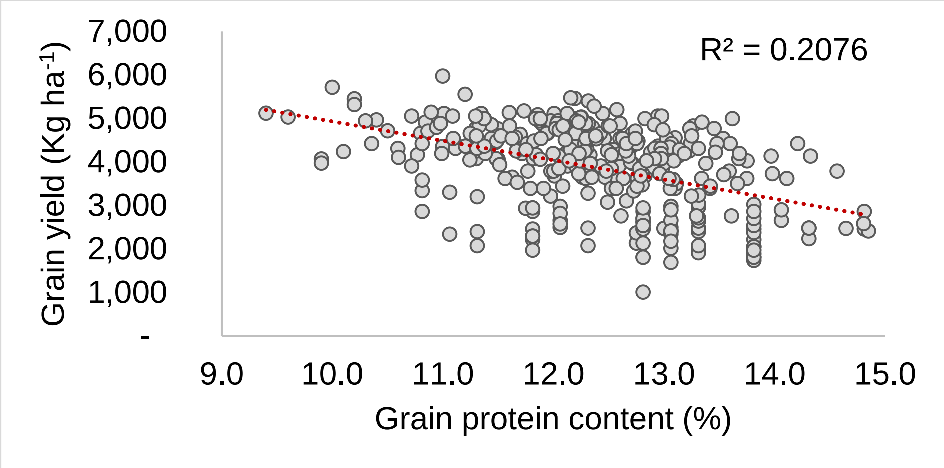


Figure 54. Negative relationship between grain yield and grain protein content (Stage 2 yield trials at Marchouch in 2017-18)

SDS, gluten index (GluInd), and mixograph (Mixo) are traits related to the suitability of a variety to be used to make breads and pasta. While strong gluten provides a clear advantage for making durum breads, the literature does not support the same trait for pasta firmness. In fact, protein quantity is found to be more important than protein quality (see Sissons et al., 2007). In fact, fresh pastas require an extensible dough with weaker gluten to improve sheeting properties. Furthermore, durum flour is very tenacious, and it is generally mixed with common wheat flour to obtain good bread volume. From a breeding perspective, it is thus critical to select lines that do not have weak gluten, but direct selection for top SDS is not needed for pasta production, although it will have an effect on the ratio of common wheat flour that needs to be mixed to produce good bread.

Three major scores are used to determine the gluten quality of durum gluten: the SDS score is used to define the fraction of proteins that are in fact gluten, the GluInd is a function of the SDS score and the GPC, and the Mixo score is the instrument of choice to predict pasta firmness. The SDS is under strong genotypic control due to allelic composition of the glutenin and gliadin, while the GluInd and Mixo score have a strong environmental component linked to protein quantity. There is extensive evidence that the protein quality in durum wheat is governed primarily by chromosome 1B, due to the presence of glutenin and gliadin encoding loci. The polymeric protein (glutenin) is mainly responsible for the elasticity of the dough, whereas the monomeric gliadins are the extensibility-related characters. LMW-GS are encoded by genes at the GluA3, Glu-B3 and Glu-B2 loci on chromosome 1, while HMW-GS are encoded by genes at the Glu-1 loci on chromosome 1, and gliadins encoded by genes at the Gli-1 loci on chromosome 1 and Gli-2 on chromosome 6. The weak gluten LMW-1 is associated with gamma-42 gliadin, while LMW-2 with gamma-45 gliadin due to genetic linkage. It was found that Mixo development time and Mixo scores are strongly correlated to pasta firmness, with stronger gluten varieties giving longer mixing time and wider bandwidth, but the effect of protein content dominates the effect. Comparison of the Mixo score with SDS and GluInd (Fig 55) revealed limited significant correlation (r^2 = 0.28) between the traits. Hence, selection for high SDS values in earlier generations will help Drive germplasm toward stronger firmness, but it is not a substitute for testing with the Mixo, since several top Mixo entries had only moderate SDS and GluInd values. In general, good varieties for making bread will not necessarily result in good pasta and vice versa, even tough lines good for both can be found.

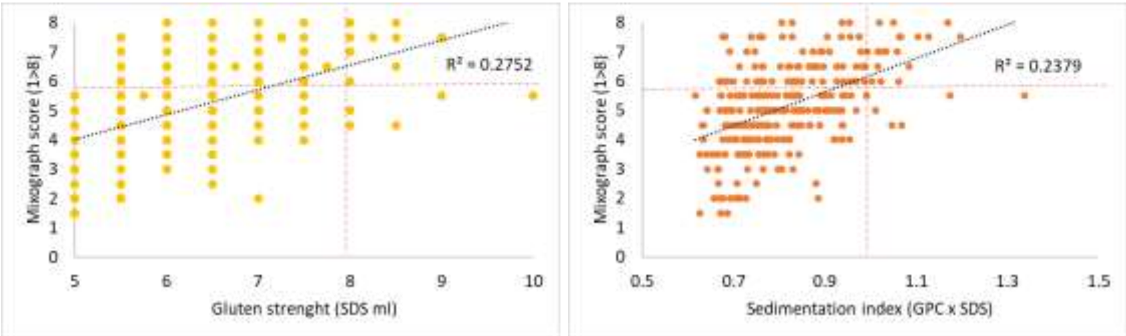


Figure 55. Mixo score relationship to SDS and sedimentation index

The dashed red lines indicate scores corresponding to top values for pasta and bread-making quality.

The literature tends to suggest that besides the importance of selecting LMW2/gamma-45 lines, the glutenin bands 6+8, 7+8, and 7+16 are all suitable for strong gluten. LMW1 and band 20 are generally considered to lead to weaker gluten. A test of 340 durum entries tested across five sites for SDS (Fig 56) confirmed a detrimental role for LMW1 and 14+15, and an advantage only for 6+8. There was a quantifiable, but not significant effect, against the use of 13+16 and 20, a mild positive effect for 7+8, and band 10, when not combined with 6+8 resulted in weak gluten. The Canadian cultivars DT570, CDC Vivid, and CDC Desire are the best donor of SDS, followed by ICARDA's line Outrob6. Some molecular markers have been made available as KASP to select for SDS (ALPa-4A ALPb-4A, and Glu-B1), but some extra work appears still to be needed to become able to select for 6+8 and LMW2. From a pre-breeding perspective, no investment should be done to seek novel alleles as all the needed diversity is already available, but some focus should be given to develop KASP markers to enable selecting the best banding pattern.

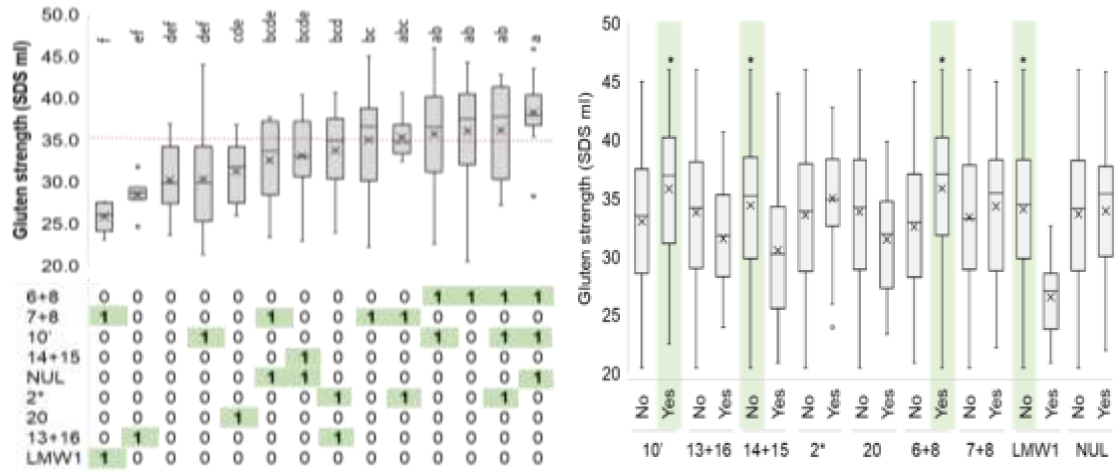


Figure 56. Combined and individual allelic effect on SDS for different glutenin bands in durum wheat tested across five environments

Whole grain, micronutrient content and fiber are three aspects of great interest for consumers looking for healthier food. The use of whole grain flour results in a loss of pasta firmness, the most critical factor for consumers. Industrial processors tend to circumvent this issue by producing semolina and then adding various ratios of bran powder. However, there might be scope to investigate genetic variation for Mixo score and Mixo time (linked to pasta firmness) using wholemeal flour to develop varieties better-suited for this new industrial target. The use of bran in commercial pasta, couscous, and several other durum foods (breads, breakfast cereals, bulgur, *freekeh*) opens the possibility to bio-fortify these products with varieties that accumulate higher levels of fiber and micronutrient. In this sense, it is important to first understand that any food product that relies on milling can easily be improved by the addition of chemical elements or by mixing with fiber-rich grains like barley.

Table 27 reports the costs for industrially-enriching flours. In all cases, these additions represent a very small fraction of the final cost of the food item and will be favored by the industry. However, for products that are made directly from unmilled grains, and for small-scale household production of food (like couscous), the option of bio-fortification is of interest. Furthermore, different food types can deliver nutritious elements at different rates (Table 28). For instance, zinc concentration increases four-fold when household foods are used instead of industrial ones. Also, the RDA indicates how much of a given element should be consumed. Setting a reasonable daily consumption of durum wheat products to 100 g per person per day means that only food products that reach 30-50% of the RDA daily dosage can be considered as nutritionally useful.

A study of CWR-derived lines across four sites with different soil types for micronutrient content (Fig 57) revealed weak heritabilities for iron (0.12), and good for zinc (0.45) and selenium (0.51). The different sites promote diverse accumulation of the nutrients, with Marchouch soils being particularly rich in iron, Tessaout in zinc, and Sidi el Aydi rich in selenium. The average highest concentration of iron was recorded as 33 mg Kg^{-1} for Zeina, 36.5 mg Kg^{-1} for zinc by IDON39-27, and 168 µg Kg^{-1} for selenium by IDON39-27. Considering the RDA of 10 and 5 mg day^{-1} for iron and zinc, respectively, and 50 ug day^{-1} for selenium, concentrations of 30 mg Kg^{-1}, 15 Kg mg^{-1}, and 150 µg Kg^{-1} should be achieved to deliver at least 30% of RDA in 100 g of food. Therefore, nutrient-charged improved lines could be identified for each micronutrient. The use of these genotypes combined with the strategic selection of cultivation area, as well as use via traditional food and the development of strategic value chains, could be used to tackle poverty in rural areas. However, since these are niche products that can only be exploited via targeted value chains, it is best to prevent further pre-breeding investments, unless specific approaches are developed to also target the markets.

Table 27. Cost calculation for flour fortification by chemical compounds

Nutrient	Source	RDA (mg/day)	Conc (%)	US$ x 1 kg source	US$ x 1 kg flour
Iron	EDTA	10.00	13.5	79	0.02900
Zinc	Zinc sulfate	5.00	36.0	7	0.00100
Selenium	Sodium selenate	0.05	42.0	52	0.00002

Table 28. Nutritional value of different durum wheat foods

For 100 g, from USDA FDA	Unit	Semolina	Pasta	Couscous	Soup	Bulgur	Freekeh
		Industrial			Household		
Energy	Kcal	360	371	376	**339**	**342**	356
Protein	G	13	13	13	**14**	12	**14**
Fiber, total dietary	G	4	3	5	15	13	**17**
Iron, Fe	Mg	4	3	1	4	3	**5**
Zinc, Zn	Mg	1	1	1	**4**	**4**	**4**
Selenium, Se	µg	70	63	65	**89**	60	**95**

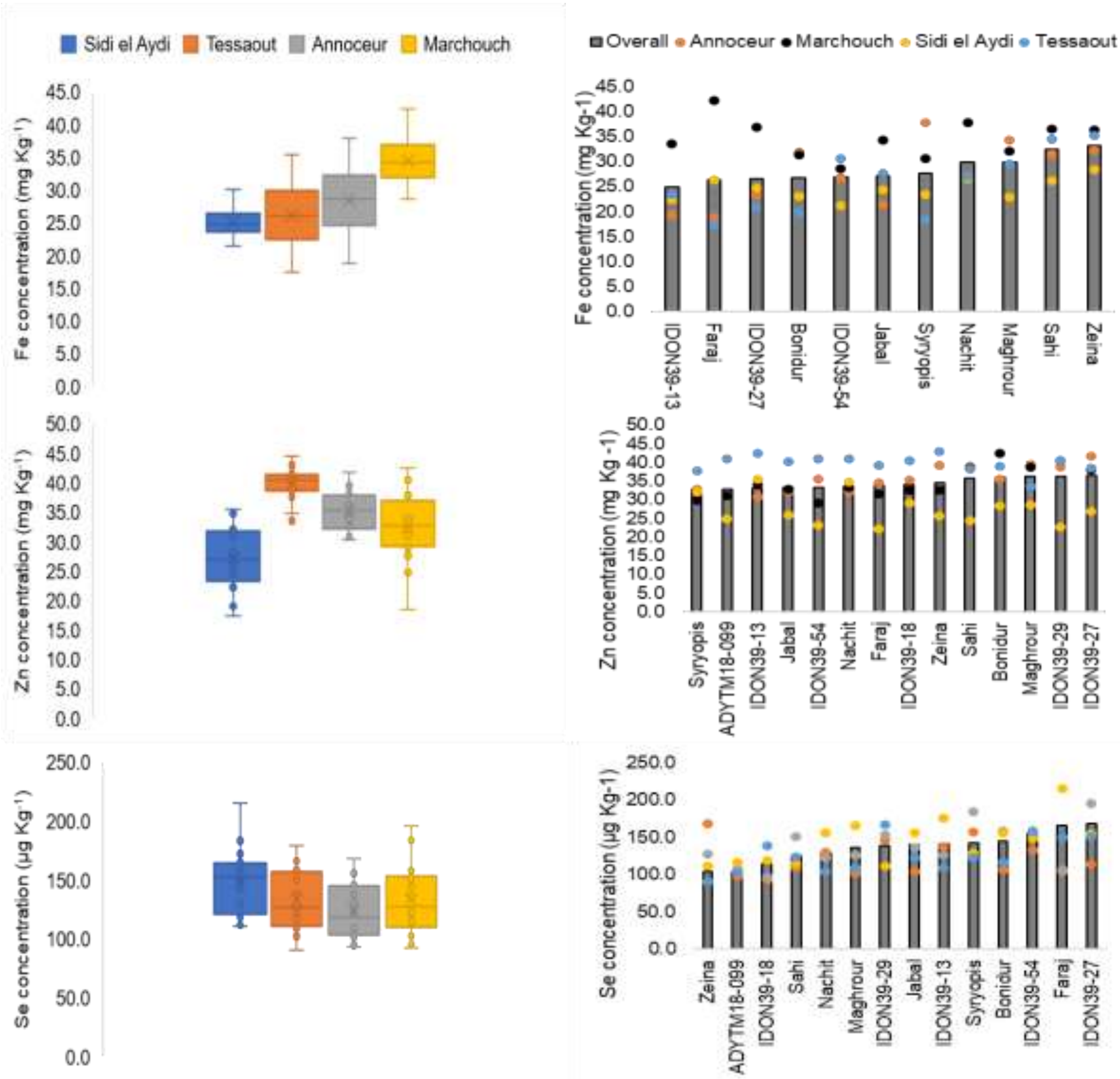


Figure 57. Micronutrient concentrations across four sites and for specific CWR-derived lines

Ancient grains are fashionably utilized to give consumers the feeling of 'traditional' and 'healthy' food products. However, landraces were never truly selected for their rheological quality, meaning that they typically have scarce pasta firmness and weak gluten. Furthermore, their adaptation is often scarce with low yields and susceptibility to several diseases. Nevertheless, landraces have often been selected over centuries by farmers for their fragrance, as this trait is in high demand among consumers.

To take advantage of the available diversity in grain, one approach is to promote landraces that are better-suited for transformation. Table 29 summarizes the results of the testing of 96 landraces across five sites. Only a few entries were found with top rheological quality, but these include ig:130807 from Sardinia (Nuoro), Italy, and ig:97186 from Irbid, Jordan. Another pre-breeding approach is to hybridize these good quality landraces with modern top-yielding germplasm, and then select for tall types with good disease resistance, top rheological quality, tolerance to lodging, and good yield.

Table 29. Summary results of rheological testing of landraces across five environments

ID (ig)	Origin	GPC	SDS	GluInd	YP
130807	ITA	18%	42.2	7.4	20.2
97186	JOR	16%	37.0	5.8	19.7
92324	SYR	16%	35.8	5.7	19.1
84294	TUR	17%	36.8	6.4	19.1
126364	ARM	15%	39.9	6.1	18.8
96156	Biadi	16%	39.0	6.1	18.7
96252	JOR	16%	41.5	6.5	18.4
96149	Biadi	16%	38.5	5.9	18.3
76851	ETH	19%	25.9	4.9	14.1
99205	YEM	17%	29.4	4.9	15.2
96259	JOR	14%	27.4	3.9	21.0
98680	CHN	14%	26.4	3.7	17.1

Organic farming is also a fashionable approach to gain consumer trust and occupy niche markets. On several occasions, ancient grains have been combined with organic farming practices. For this, the same approach described above can be used, but this time testing needs to occur under zero chemical inputs. Several key traits of organic farming conditions common to those of 'stressed' environments. These include rapid ground coverage to compete with weeds, strong NUE to take advantage of organic nitrogen in the soil, and good resistance to abiotic stresses. Pre-breeding investment in this regard should be sought only if accompanied by specific supporting projects and market opportunities. Smallholder farmers in ICARDA's target region practice some forms of organic farming, but typically provide some fertilizer inputs and apply fungicide in the case of strong epidemics.

Special traits

Farmers' preferences vary widely across the ICARDA's targeted region and are dependent on many traits that are seldom captured by breeders. Nevertheless, the ability to capture these traits to include in varieties can ensure gaining large market shares. Here is a short list of traits, which are part of 2019 PP agreements:

Black awns are a characteristic trait of the durum wheat cultivar (Fig 58). Only a small fraction of commercially cultivated cultivars have black awns by the end of the cycle. Still, farmers in North Africa and West Asia have a clear preference for this spike type as it is widely present in landrace populations. A survey conducted with various farmers as part of participatory variety selection confirmed a strong preference for this trait to the point that farmers often preferred candidates that were not top yielders but showed black awns. There is limited literature on the subject, but it is empirically evident that the trait is under genetic control, with the environment affecting the level of pigmentation expressed. The recent release Nachit (syn. Trouve) is a good source of this trait, and more than 30% of

ICARDA’s germplasm is also characterized by black awns. In that sense, there does not seem to be any need to invest in pre-breeding activities to seek parental donors for this trait, but it would be of interest to determine the genetic loci controlling it. A simple one season study on a mapping population (DRO-MP) that was segregated for Jenaa Katifa’s black spike color (Fig 59) revealed the presence of a major QTL or QTLs effect on chromosome 1B.

Figure 58. Special traits (from left to right): black awns, red straw, and awnless type

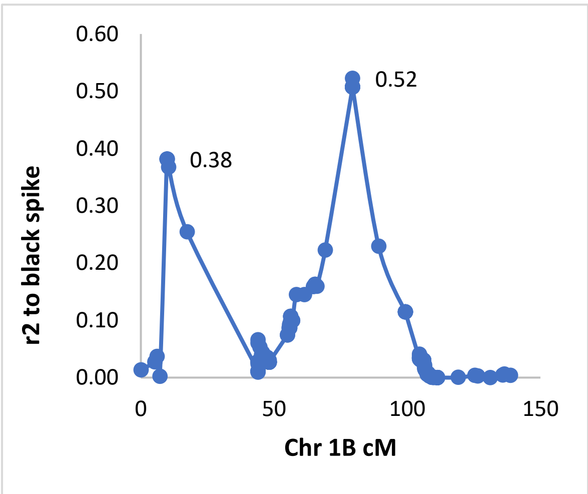


Figure 59. Major QTL or QTLs on chromosome 1B in the DRO-MP mapping population originating from a cross with Jenaa Katifa with its characteristic black spike color

Red straw color is a very peculiar characteristic that makes certain durum wheat varieties stand out (Fig 58). ICARDA’s first release Waha (syn Cham1) is a good example of this trait, but also the Tunisia landrace Jenaa Khatifa carries it. This characteristic is of great interest for Algerian farmers who have cultivated Waha for many decades. Similar to black awns, this trait is under genetic control, with Waha derivatives normally showing the characteristic pigmentation. Also, Margherita and Icaverve tend to have the same reddish characteristics. Pre-breeding efforts do not seem justified for this trait, but genetic dissection could be of interest.

Awnless is a unique type of spike that has found great appreciation among common wheat farmers in the UK, but that is almost completely absent from the durum catalogues. The Australian cultivar Saintly is an exception with its spikes showing pointed barbs instead of awns (Fig 58). This rather minor trait became of great interest when empirical evidence suggested that it might actually contribute to reduced bird damage before the plant reached maturity. Small birds feed by grasping the awn in their beak, then let themselves fall, and this gesture pushes the seeds to come out of the glume and Drop to the soil. Testing of Saintly in zones with a strong bird presence (nearby trees, during summer cycles, or along the Senegal River) revealed, in fact, a much lower level of damage. For that reason, Saintly was hybridized with several ICARDA elites, and awnless spikes were selected. Since this trait is of interest only under specific conditions and good donor lines have already been introgressed with ICARDA germplasm, pre-breeding activities do not seem to be justified.

General strategy for pre-breeding

Pre-breeding is a core activity of the CGIAR breeding program. The ability to collect, tame, exploit and understand genetic diversity is a public effort that has been recognized over the years. For any pre-breeding effort, four major steps are normally taken:

1. Identification of a target trait in need of pre-breeding;
2. Testing and discovery of germplasm carrying useful alleles;
3. Direct exploitation into breeding;
4. Genetic dissection of the trait of interest to simplify the breeding selection.

1. The first step requires forward thinking to identify problems that are or will become important in the near future (5-10 years), but also to be honest about the possibility of solving them via breeding or integrated approaches. One good example is disease resistance, with many fungal pathogens that are commonly controlled even by smallholder farmers with fungicide applications at costs of just US$20-50 per ha. It is therefore good practice to seek moderate levels of genetic resistance that can then be complemented with fungicide use. It is not the case for those diseases that occur after flowering (when fungicide use is very difficult) or for which the treatment is ineffective, like fusarium.
2. The second step is carried out by many programs around the world, so it is critical to invest only in novel discoveries that are not already well complemented by others. Thorough scouting of the literature is important to find partners or already available donor lines. A critical step of testing is access to germplasm, but also development of good phenotyping methodologies. One critical outcome of this step is the identification of donor parents for hybridization and of potential proxy traits (for instance TKW for drought tolerance) that can be immediately deployed in breeding.
3. In step three, the donor germplasm needs to be immediately picked up by CGIAR breeders and used for hybridization with top performing elite lines. The proxy traits

need to be used for selection to try and quickly deliver adapted elites with the extra trait that can be distributed to partners.

4. The genetic dissection is, in many cases, a secondary step. Breeders will not wait to know what QTLs or genes are involved in controlling a specific trait before using the donor parents in their program. Still, genetic dissection can help guide introgressions in the following stages. For alleles that can be found in several entries, genome-wide association mapping is a strategic tool that can be applied at step 2 (test and discovery of germplasm) to identify the number and distribution of loci controlling a trait. From a breeding perspective, this step can help in determining the best donor parents to use, being those harboring different QTLs for the same trait. Also, GWAS discovery needs to be quickly converted into easy-to-use KASP markers that need to be validated on elite germplasm and scaled for MAS. For traits that are unique to specific germplasm entries, only QTL analysis of mapping populations can be used. In this case, step 3 is conducted between the donor parent and modern germplasm. The progenies are then advanced to F_4 via selected bulks, and at this point 200 individual spikes are collected. The F_5 spikes are planted in rows and DNA is collected for genotyping. The row is bulk harvested and the $F_{5:6}$ seeds are used for phenotyping. This way, QTL analysis can be conducted. The same principle can also be used to fine-map QTL of great interest identified via GWAS. In some cases, the trait and QTL associated are of even greater interest to promote an attempt to clone the functional gene controlling it. The principle of bulk-sequencing is then best applied. The F_5-derived RILs can be a good starting point for this task if the donor parent only carries one major QTL. Alternatively, the F_5-derived RIL need to be selected using marker and phenotyping to find progenies that harbor the positive allele at a single QTL. These could be converted to near isogenic lines by backcrossing or directly used for creating point mutants by EMS mutation. Phenotyping of these mutants should allow for the identification of lines that have lost the targeted allele, and once again the principle of bulk-sequencing can be used.

Each one of these steps requires targeted investment, which is why the prioritization of pre-breeding targets is such a critical step. A prioritization of traits is presented in Table 30, while a schematic of the investment process is provided in Figure 60**.** Independently of the priority assigned, some traits require less budget than others. For instance, Hessian fly is a qualitative trait for which germplasm and markers are available, and phenotyping is cheap so achieving the best return on investment will require less budget. Drought tolerance is a quantitative trait that requires several field trials to be studied. Reaching the best return on investment would require a very consistent portion of the pre-breeding budget.

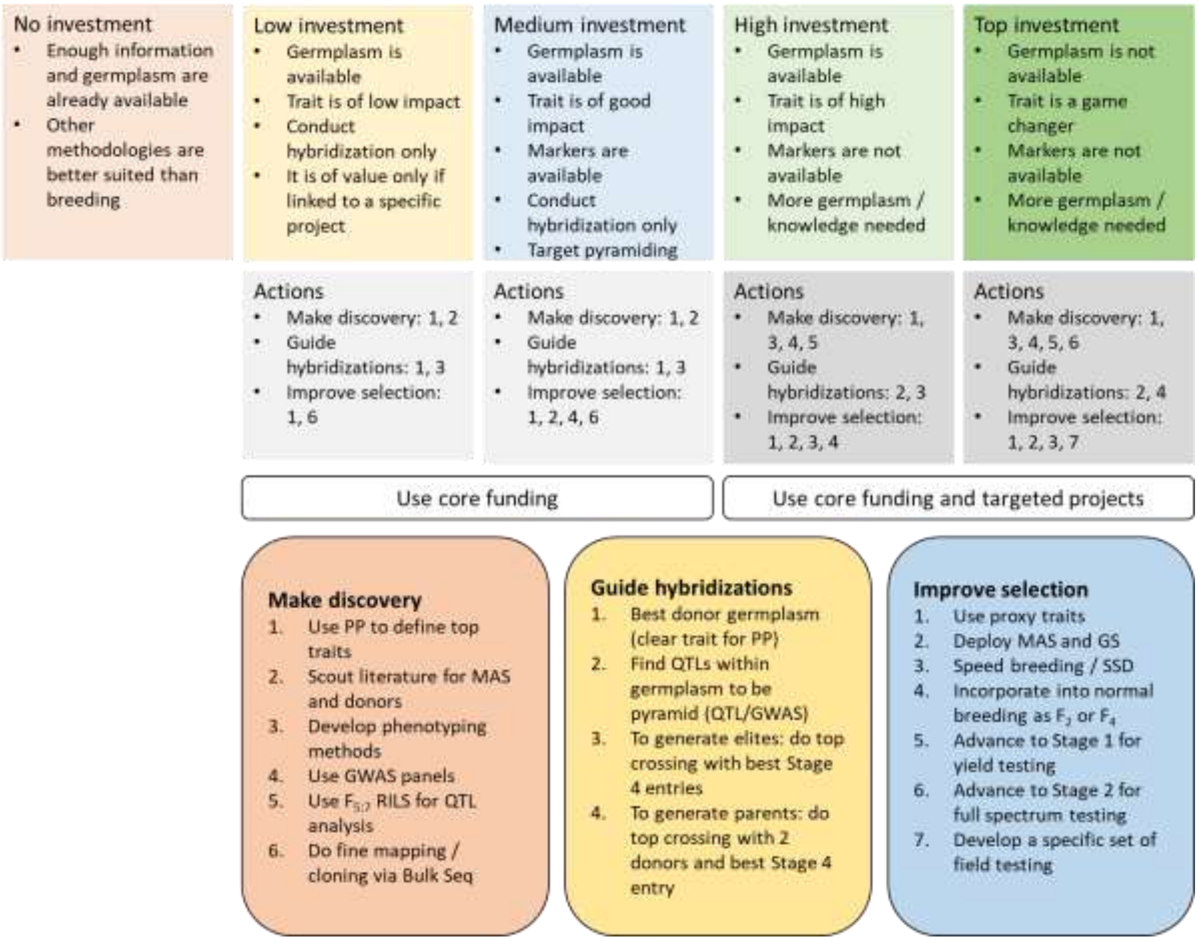


Figure 60. Schematic representation of decision process for which pre-breeding investments to make

Table 30. Pre-breeding trait prioritization for 2020-2025

Trait	Donor	Pre-breeding strategy	Budget
Crown/root rot	SSD1479-117, Icavervem Berghisyr, Marouane, Faraj, Outrob, R69-9/R5, V15x49, V15x39, V15x44, V15x86, V15x42, V15x82	Combine the resistant sources and develop markers for them. Fund INRA activities to screen Stage2 in glasshouse.	20% Top
Drought tolerance	Isly, ADYT97, Magrour, Colosseo, IDON37-52, ADYT46, Bezater, Ouassara, Jabal, ig:99205, 94615, 99261, 85404	Continue to dissect the genetic basis of tolerance. Pyramid tolerance QTLs with different root types. Use landraces to improve TKW and spike length.	15% High
1,000 kernel size	Nachit, Gigamor, Jabal, Koronos, Sahi	Use seed scanner to combine long (Jabal) and wide (Sahi) grains. Introgress TKW from emmer and pollonicum. Combine different QTL from Kronos. Define markers using MP-Syr RIL and GWAS.	15% Top
Hessian fly	Gigamor, Icaqinzen, Younes/T.dicoAlpCol/Korifla, tilling	Marker BS00072387 for HFArat, H31 in several entries, Hdicoccoides, and tilling. Fine map HFArat, pyramid resistances.	10% Top
Heat tolerance	Faraj, Kunmiki, Berghouata1, and Ourgh	Markers available and validated. Pyramid QTLs for tolerance. Use CWR to increase grain number per spike.	10% High
Leaf rust	Tunsyr, Geromtel, IDON44-12, IDON43-05, Sebatel, Gaza, Arnacoris, Saragolla, Amria, Byblos	Several markers are available: KASP markers usw222, usw215, usw218, CIMwMAS0135. Introgress APR genes from modern germplasm (Lr34, Lr46, Lr67, Lr68, Lr74, Lr75, Lr77, and Lr78). Continue seeking novel alleles.	8% Medium
Stem rust	Sebatel (Sr2, 13, 22), Arina (Sr56), Kronos (Sr13), IDON44-12 (Sr25), Cham1 (Sr 35), RWG35 (Sr47), RWG38 (SrAes7t)	Markers available: csSr2, Sr2_ger9 3p, Sr13F/R, Sr22 cssu22. Introgress what already available. Develop good KASP markers for the complex Cham1/Sebatel.	5% Medium
Septoria tritici blotch	Ouassara, Hessept, Chicca, Canzone, SWAlgia, and Helab	Seedling and marker test to determine which genes are available within ICARDA's germplasm.	5% Low
Phenology	Ouassara for early, Miki3 and Europe for medium, PI376509 from Romania for "true winter"	Need to improve marker predictability for durum. Seek 'true winter' for adaptation to future environment.	1% Low

Lodging tolerance	Landraces and Om Rabi derivatives	Test at Allal Tazi and Melk Zehr with high seeding rate. Continue introgression from landraces and select against lodging.	1% Low
Yellow rust	Most of ICARDA's germplasm	Asses several elites at seedling to confirm type of resistance is not monogenic.	1% Low
FHB*	Shahba, 10LND791, 15PRO343, RRR/Karur	Combine the three sources of resistance and work with private companies to test for FHB.	1% Low
Winter hard*	PI376509 (Romania)	Make cross and select for winter durum program.	1% Low
Yellow pigment	Sahi, Tamaroi, DBA Aurora,	Investment: integrate selection for grain color. Use markers like Lpx-A3, Psy1-A1l, and Lpx-B1.1b.	1% Low
SDS, GluInd, Mixo	DT570, CDC Vivid, CDC Desire, Outrob6	Select for 6+8 and LMW2 and develop markers for it. Use markers ALPa-4A ALPb-4A, and Glu-B1.	1% Low
Wholegrain and micronutrients*	Zeina, IDON39-27	Test whole flour from Mixo. Continue screening for micronutrients. Need to link with whole value chain of traditional foods.	1% Low*
Ancient grains*	ig:130807 from Sardinia, ig:97186 from Irbid, Jordan	Hybridize these with modern varieties, select for tall types with disease resistance.	1% Low*
Organic farming*	ig:130807 from Sardinia, ig:97186 from Irbid, Jordan	Hybridize these with modern varieties, select for tall types with disease resistance, under zero chemical input.	1% Low*
Black awns	Nachit and others	Select for in segregating populations. Test genetic of trait in MP.	1% Medium
Awnless spike	Saintly	Select for traits. Score actual bird damage in bird-infested sites.	1% Low
GPC and yellow berry	-	Utilize agronomy instead.	0% No
Red straw	Waha, Margherita, Icaverve	Select for red straw in segregating populations.	0% No

*Investment might change based on project linkages

Storage policy

Logistics is one of the most critical aspects of a successful breeding program. While in general, all tested germplasm is of potential importance, the ability of a breeder to discard what is less likely to succeed and keep only what generates long-term gain is as critical as any other steps of the program. Average size durum wheat programs harvest 20-40 ha of breeding material each year, at an average yield of 3-5 ton per ha, equaling nearly 100 tons of grains per season. These are then packed in small bags and envelopes of just a few kg. Handling such a vast set of entries would require enormous investments in seed storage facilities. Furthermore, the average germination rate of durum wheat grains Drops quickly as moisture and temperatures exceed certain levels. To ensure 90% germination, durum seeds can be stored at max 15% moisture and max 25° C for up to 1 year, at 13% moisture and max 20° C for up to 8 years, at 12% moisture and 4° C up to 20 years, and over 70 years at 10% moisture and -20° C temperatures. As storage conditions become more challenging, so their cost increases. Breeders need then to really define what type of germplasm needs to be stored under which conditions.

General rules of storage: containers

The enemies of seed vitality are damaging pests, moisture, temperature, light, and human errors. Pest are capable of easily penetrating paper and plastic tissues even after just 1 week of storage, but are unable to enter glass jars or plastic barrels. Hence, all storage that exceeds 1 year must be made inside sealable plastic barrels with naphthalene pills to protect against pests (Fig 61). The glass jars are used for 2-3 years' seed storage, which can be displayed to visitors and farmers. Small 1-2 l barrels can be used to store pure seeds of varieties for up to 5 years, or 500 spikes for 1-2 years after fumigation, while larger sealable barrels are used for 5 years storage for specific trials.

Figure 61. Storage containers: glass jars for display, small barrels for individual varieties pure seeds or spikes, larger barrels with locks for long-term storage

General rules of storage: labels

The label is as important as the storage container. It is very easy to lose trials in a full seed storage, and as easy to forget the reason they were stored. Hence, very clear labels need to be used. An example is presented in Figure 62**.** The principle is that anybody in the team can be absent without impeding the ability of others to retrieve the same seeds without issues, or for the seeds to be discarded whenever needed. The label needs to contain basic information, such as the trial, the entries, and other details based on the program needs. The most critical aspect that is often forgotten is the date of storage and the date of discard. Adding store position will help with easy location of the seeds whenever needed. A QR or barcode can further help to keep track of information electronically, including the amount of seeds stored.

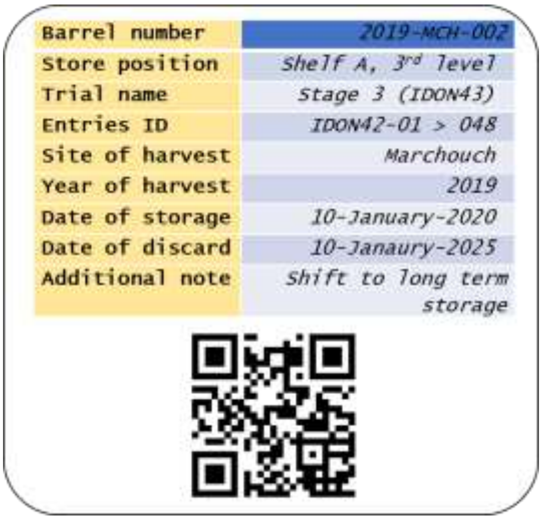


Figure 62. Example of a label used on storage barrels

General rules of storage: storage time

At the end of each season, the grains are harvested, analysis conducted, and new sets are prepared for the following season. After planting is completed, the season calms down and the staff has time to work on the storage aspects. The first step is to discard any stored seeds that have reached their expiration. The second step is to put new steps into store following the principles below.

Temporary storage (12 months): this is the most common form of storage and is applied to all germplasm that will not be used again. Because seasons are unpredictable, breeders prefer to discard seeds from the previous season only after the ongoing season is concluded. A fire, a drought, or any other possible natural disaster could potentially wipe out all planted seeds in the field, creating an absolute need to go back to the seeds from the previous season. This storage is practiced inside the harvest bags kept directly on Dry surfaces (pallets) at 15% moisture and less than 25° C. Fumigation is applied at the time of storage and after 6 months. For the ICARDA program, the following sets are discarded after 12 months:

- Segregated populations F_1 to F_5;
- All Stage 1 and Stage 2 trials;
- Stage 3, 4, and 5 trials from sites other than the main stations (Marchouch or Terbol);
- Academic study sets (association panel and mapping populations) from sites other than the main station;
- Non-pure seed productions used for demonstrations;
- Spikes harvested for purification.

Short-term storage (60 months): this is an expensive form of storage and is applied only for germplasm with high value, or for which new, interesting information can be generated in the course of a 5-year span. This storage involves the weighing out of exactly 250 g of seeds, which is deemed enough to quickly produce 10 kg in just one season if the need arises. The seeds are stored inside labeled plastic bags, moved into plastic barrels conserved at less than 13% moisture and max 20° C. For the ICARDA program, the following sets are stored:

- Original seeds of academic studies (association panel and mapping populations);
- Stage 3 IDON, Stage 4 IDYT, and Stage 5 from one site;
- Crossing block entries not planted the following season;
- Pure G1 seeds of candidate varieties;
- Breeder's seed (G3) of candidate varieties.

Medium-term storage (120 months): this is a very expensive form of storage and is applied only for germplasm with recognized present and future value. This storage is practiced by weighing out exactly 10 g of seeds. The seeds are stored inside vacuum-sealed aluminum bags, moved into plastic racks in the fridge at 4° C. For ICARDA's program, the following sets are stored:

- Original seeds of academic studies (association panel and mapping populations);
- Stage 3 IDON;
- Pure G1 seeds of released varieties.

Genebank storage (perpetuity): this is a special form of storage and requires the approval of the Genebank manager. After 10 years of storage in the fridge, the seeds are grown out in a glasshouse. Data are gathered for each and presented to the Genebank manager. A case is made for *in perpetuity* storage and, in exceptional cases, the Genebank accepts to include the seeds into their vault breeders' germplasm, which is part of the multi-lateral system and be available for use forever.

Impact assessments

Another important step that does not always receive enough investment by breeders is the continuous assessment of progress in the form of market shares, appreciation, release of varieties, and genetic gain. This section describes how these different aspects are handled by the ICARDA breeding program.

Increase in market shares

The ultimate goal of breeding impact is to measure what percentage of the targeted area is actually cultivated with varieties originated by the program. Ideally, genotyping-based surveys provide the best information on which varieties are cultivated, but these surveys are expensive and time-consuming. Instead, a report on annual seed production and sales from the main national seed enterprises is an excellent proxy and is easier to obtain.

Alternatively, national breeders' opinions are also valuable to capture progress regarding various factors. Figure 63 reports the percentage of market share occupied by different ICARDA varieties as of 2016, based on breeders' opinions.

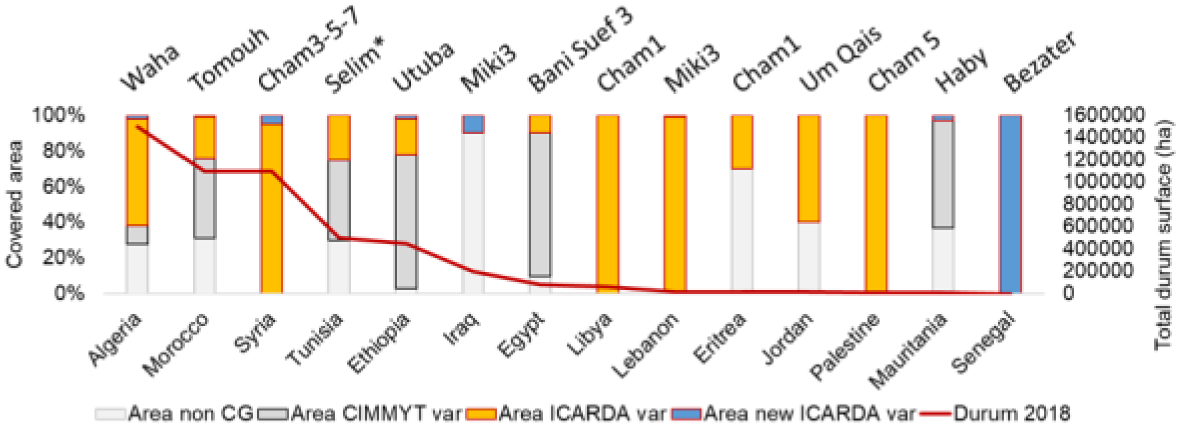


Figure 63. Market share occupied by ICARDA varieties in 2016 based on national breeders' opinions

ICARDA's main responsibility is to provide germplasm and facilitate variety release. In that sense, a good measurement of the program's impact is the number of clients that request the nurseries year after year (Fig 37) and the number of varieties released (Fig 5 and Fig 64). There has been a positive trend in the number of requests of Stage 3 and Stage 4 germplasm, which increased from 30-35 requests before 2012, to 100 in 2015, and has since stabilized to around 75 per year. More importantly, there is a large number of partners that keep requesting and testing the germplasm year after year, including some European private companies, which indicates good performance and overall usefulness.

In terms of releases, there are 144 confirmed variety releases to date in 23 countries. The greatest historical partners of ICARDA have been Algeria, Syria, Morocco and Turkey with 24, 17, 16, and 15 releases each respectively, then Lebanon, Ethiopia and Iran with 10, seven, and six. With the exception of Cyprus, Greece, Libya, Portugal, Saudi Arabia and

Spain, and all other countries have continued to release the varieties in the past 10 years. Of particular note is the work carried out by Azerbaijan, which has steadily released new ICARDA-derived varieties every 2 years since 2016 when it first started.

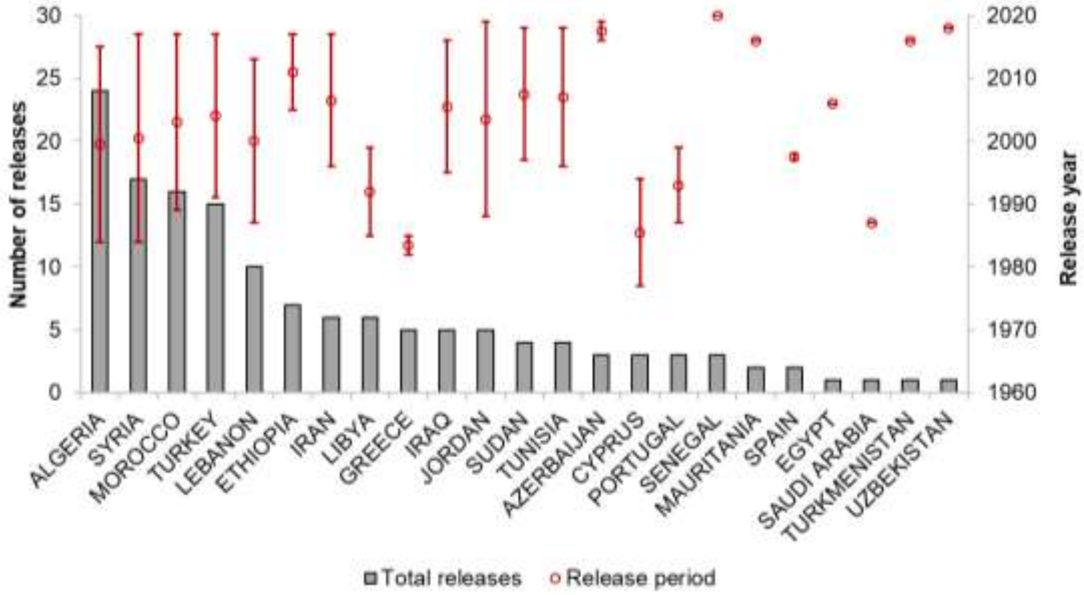


Figure 64. Number of varieties released per country (grey bar) and period of release (red whisker)

Genetic gain

The genetic gain is considered by many breeders as the true value of a program. Variety release and commercialization are subject to several external factors rarely under the control of the breeder, while genetic gain is solely due to the ability of the program to progress. Genetic gain can be measured based on several traits, but ultimately, grain yield is the trait that best summarizes progress, which can be measured in several ways. The basic principle is to reduce the GxE effect to the minimum in order to see G, and then compare different ages of germplasm. At ICARDA, it is deemed that the crossing year is the most reliable and stable approach for this type of study. Alternatively, it is possible to compare trial ages (i.e. Stage 1 vs Stage 2 vs Stage3 vs Stage 4) or the same trial over years (i.e. Stage 4 of 2019 against Stage 4 of 2018). The most challenging is to compare between release years of cultivars, since this is also influenced by many factors external to breeding and it is normally referred to as the 'realized genetic gain'. What is not acceptable is to compare pears to apples: one common error is to compare release year of older cultivars against the trial year or cross year of more recent elites. Such an approach is sure to boost the genetic gain, but it is highly unfair.

Every 5 years, the ICARDA program conducts a comprehensive assessment of its genetic gain since inception, which involves growing side-by-side across-site top germplasm developed during the history of the program against the most recent elites. In a study by Bassi and Nachit (2019), 35 years of ICARDA's history were compared across 10 environments and seven sites. Decade-wise genetic gain for grain yield was measured to reveal >9% (equal 0.9% per year) at eight of the environments, while no progress was found for the Dry and cold conditions of the Atlas Mountains. Combined analysis revealed an average genetic gain per year of 0.7%, mostly Driven by earlier flowering and an increase in spike density. This achievement came at the cost of eroding 6.3% of the original genetic diversity, and more than a three-fold reduction has occurred for rare alleles (Fig 65). The study also indicated a reduction in TKW, which prompted a rapid intervention in this sense.

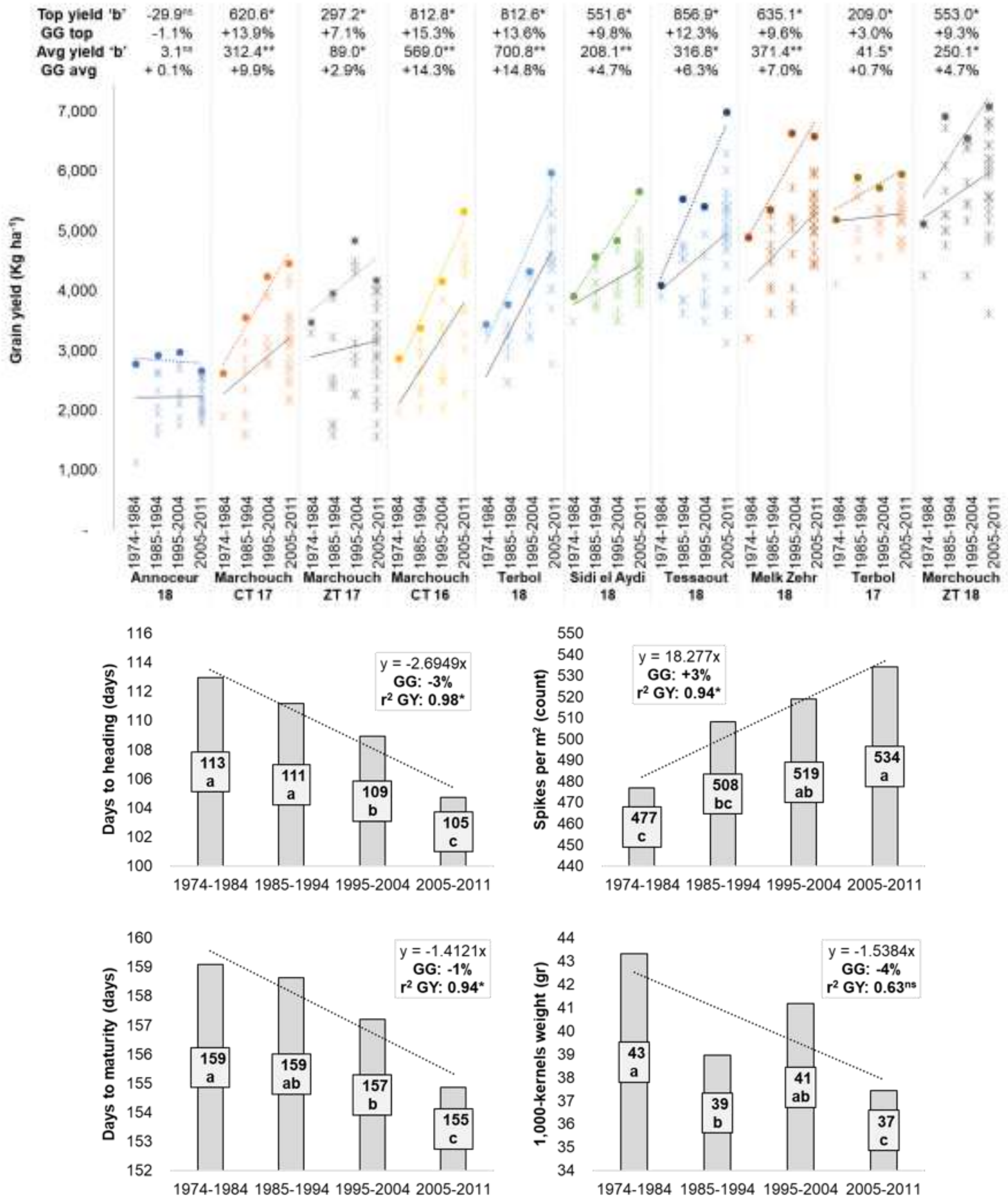


Figure 65. Decade-wise genetic gain of 35 years of ICARDA's durum wheat breeding

While this is certainly the clearest way of assessing progress, it is not possible to conduct specific genetic gain studies each year. Instead, simplified comparisons are used for monitoring annual progress. A small subset of 24 entries are selected from the previous work to represent the major progress of each decade. These are planted annually in two reps in an alpha lattice design next to the Stage 4 trial, with the same plot size and planting date. Annual progress can then be monitored across key sites as reported in Figure 66. In the example for 2019, it is clear that no progress was made for Terbol, while +0.48% for Kfradan and 0.14% for Marchouch was achieved. These are low rates of genetic gain but can be explained by the fact that all sites received late rains, which favored late-flowering genotypes.

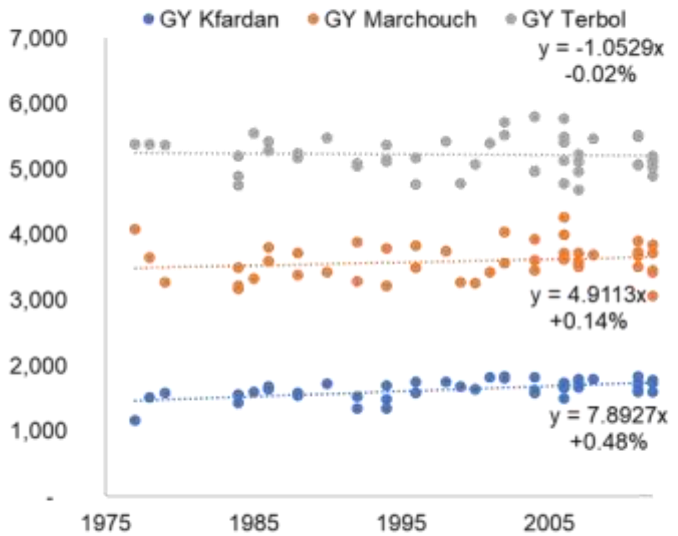


Figure 66. Annual monitoring of genetic gain at three key sites (genetic gain set against Stage 4)

From a CGIAR prospective, it is probably of greater interest to see how the Stage 4 trials performed at the national partners' sites. Since stations and agro-climatic conditions change every year, the best approach is to report the performances as ratio to the commercial check provided by each national partners. Then Stage 4 trials of different years can be compared for their yield stability (AWAI) and yield potential (yield combined across sites). The result for 2019 is reported in Figure 67. Since it is a biplot approach, the genetic gain needs to be presented as the number of entries matching the best performances. It can be seen that IDYT38th had good stability and yield potential with 75% of entries surpassing the check and the best entry overall.

IDYT39th was the most yield-stable set, but had little yield potential. IDYT40th was less stable, but was high in yield potential with 50% of the entries superior to the check for both traits. IDYT41st had increased stability and good yield potential, with 30% of the entries superior to the check. Finally, all entries for IDYT42nd had better-yields than the check and were stable, with the second-best entry overall. This trend is in line with the issues encountered by ICARDA after their movement out of Syria, which meant Stage 3 and Stage 4 sets were produced from older crosses, and a larger portion of older germplasm had to be recycled. Hence, the first nursery selected was the best and the following were initially poor but progressively got better, until 2019 when IDYT42nd was overall the best nursery of the previous 5 years.

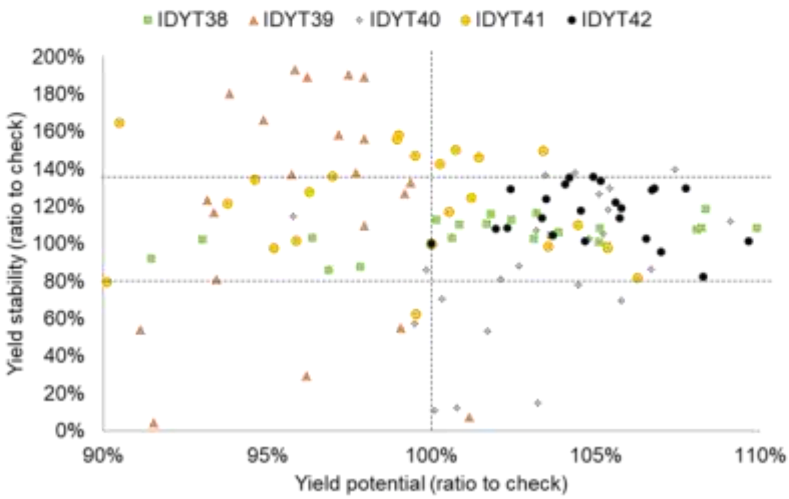


Figure 67. Comparison of Stage 4 (IDYT) trials of different years across sites

The final and most simplistic way to check the annual rate of genetic gain for grain yield is to compare Stage 4 vs Stage 3 vs Stage 2 yield trials during the same season at the same site. This is often defined as the potentially attainable genetic gain, since it is very hard to record it again once all entries are planted within the same experimental design. Figure 68 represents the comparison for three yield trial stages at two sites (Marchouch and Terbol). The average genetic gain is of lesser interest for this type of approach since the entries of Stage 4 are better selected than for Stage 3 and Stage 4, but it is positive to see less dispersion in grain yield as the stages progress. Instead, the most interesting result is that for top yield, in the example, there is 29% potential gain for grain yield between Stage 3 and Stage 4 at Marchouch, and 2% between Stage 2 and Stage 3. This is an excellent indication of potential progress.

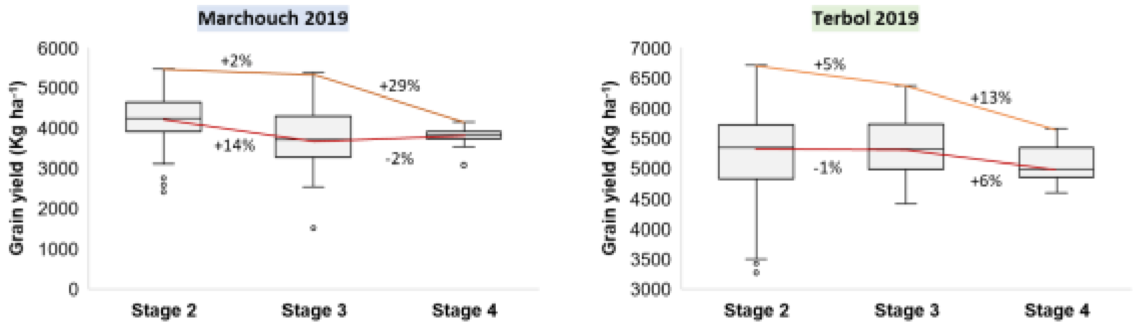


Figure 68. Genetic gain measured as a comparison of yield trial stages in the same environment

Data are presented for Marchouch and Terbol 2018-19 season.

The most comprehensive way of determining the progress of a breeding program towards the achievement of its goals (PPs) is to present Stage 4 nurseries as PP matching. This is calculated as the multiplication of the index score, yield at the TPE, and correct phenology. Matching of 70% or above is deemed a good result that should satisfy the clients (national breeders in this case). Figure 69 reported the results for Stage 4 across 14 PPs. For nine PPs, good matching was achieved; for two (Ethiopia lowlands and India 1, 2 irrigations) matching reached only approx. 60%, so more work is still required, but the germplasm is responsive.

For three PPs, poor matching was obtained – the main reason for two of these being the required phenology of facultative winter types, which has been addressed by the development of a separate facultative winter program. For the Senegal River, it is clear that more efforts are required to integrate strong gluten alleles into heat-tolerant germplasm.

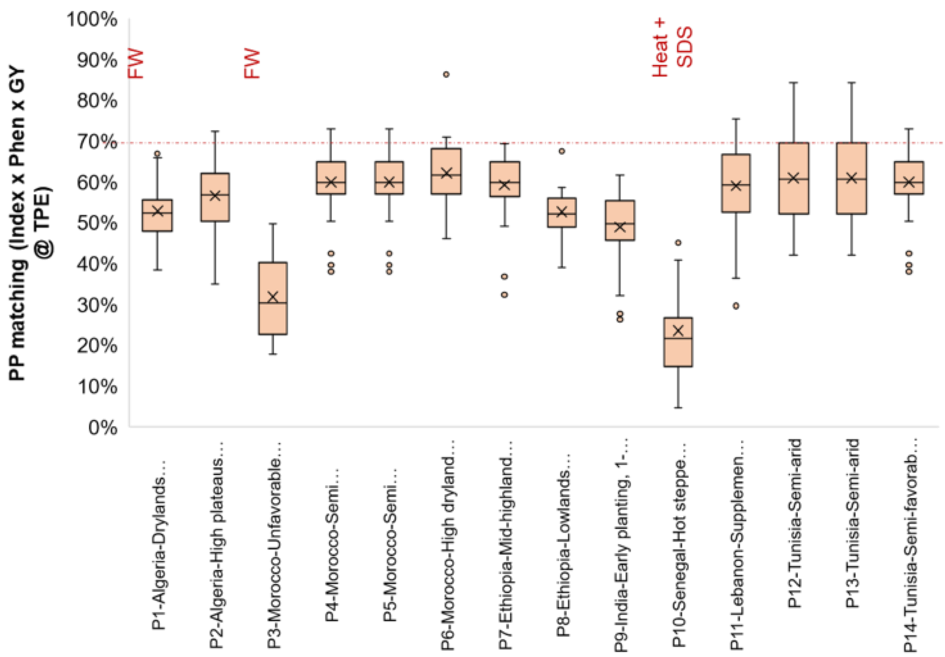


Figure 69. PP matching of Stage 4 germplasm in 2019

Comparison to competitors

Ultimately, the most critical assessment of a breeding program capacity to have impact is an objective comparison to its competitors. In the case of ICARDA, there are three types of competitors: 1) the national check cultivar used by national breeder to evaluate the performances of Stage 3 and Stage 4 international nurseries, 2) national and international private seed companies, and 3) other CGIAR institutions.

The comparison to national check is done annually by recovering data from Stage 4 yield trials. Figure 70 reports the partial results obtained in 2019 for IDYT42nd (Stage 4). It is possible to observe that in all 16 sites, the national check was out-yielded by one or more entries from ICARDA. A 32% average grain yield advantage of IDYT42nd over check across environments was identified, with 10/16 sites showing statistically significant superiority. A 19% yield advantage was also recorded for Spain and Turkey, 15% for Greece, and 50% in India under one irrigation. This type of measurement ensures an indirect comparison to the ability of national public breeders to deliver superior germplasm, and to guarantee that the Stage 4 trials of ICARDA remain useful.

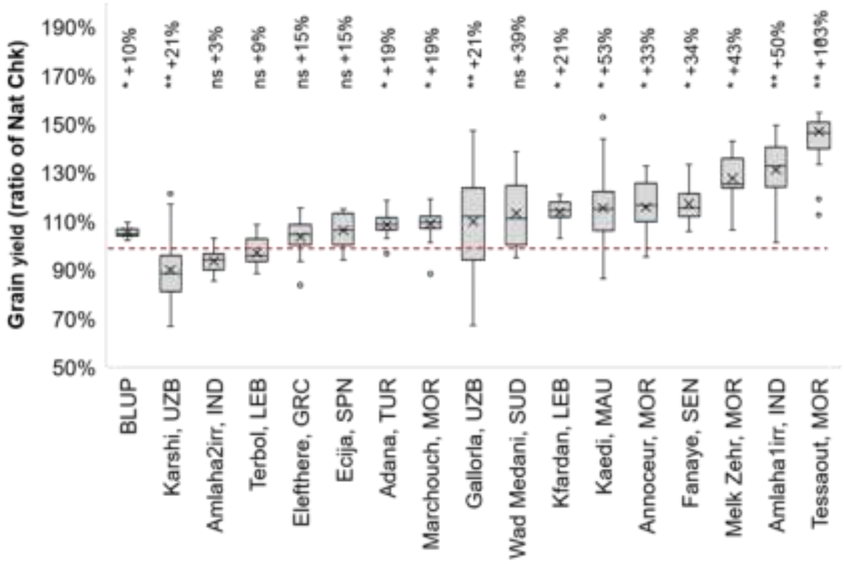


Figure 70. Yield comparison to national check for grain yield of Stage 4 trials in 2019 across 16 sites

The major competitor for any public breeding institution is the work carried out by private seed companies. Investment into public breeding cannot be justified if results are inferior to the work carried out by private seed enterprises. For this scope, Stage 5 trials are run to incorporate ICARDA's best germplasm, national partners, and private seed companies. Figure 71 is an example of a comparison between three Moroccan cultivars (Karim, and ICARDA's Faraj and Nachit) against germplasm provided by national breeders (INRA), a private company (Florimond-Desprez), CIMMYT and ICARDA. Across four sites, ICARDA varieties proved to be the best. ICARDA elites were also top at four sites, with Marchouch showing matching to the private company. CIMMYT was the best under the ferti-irrigated conditions of Melk Zehr. These results confirmed the good quality of ICARDA's germplasm, but also the fact that private company germplasm is extremely competitive under various conditions and should hence be incorporated as a check in Stage 2 trials and in the crossing block.

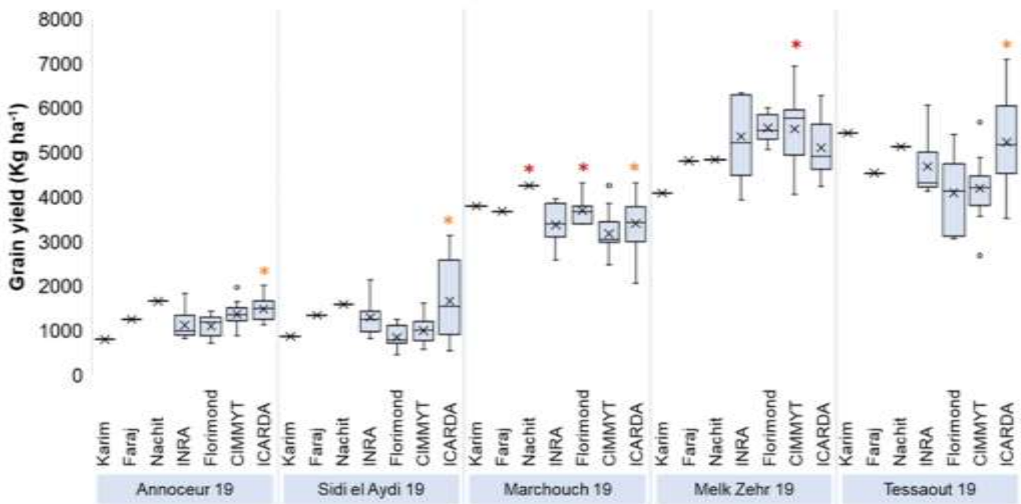


Figure 71. Yield comparison of ICARDA competitors across sites of Stage 5 yield trials

ICARDA’s main competitors have historically been the CGIAR center CIMMYT and the Arab Center for the Studies of Arid zones and Dry lands (ACSAD). CIMMYT resembles the ICARDA program in size, strategy, and scope, and it has been equally successful in delivering varieties to farmers. Under the CRP WHEAT initiative, the two centers have re-initiated a much tighter collaboration to work together to achieve common goals. Still, to justify their co-existence it is necessary to show true distinct goals and synergetic complementarity, with ICARDA primarily targeting Drylands and CIMMYT better suited to achieving higher-yield potential under high moisture. ACSAD conducts a smaller breeding program with a reduced geographic target, and with less varieties included in the catalogue. Also, since ICARDA and ACSAD were previously based in Syria, the two centers have exchanged a wide selection of germplasm and many of the ACSAD varieties today carry ICARDA pedigrees. In that sense, ACSAD and ICARDA were never truly considered as rivals, and it remains important to continue this partnership and measure comparative progress.

One of ICARDA’s primary targets are environments (TPE) represented by the site of Marchouch in Morocco. A 5-year side-by-side comparison of Stage 4 trials (IDYT vs IDYN) from CIMMYT and ICARDA revealed that, for 4 years, ICARDA’s nurseries were superior for top and average yield, while in 2014, the set IDYT39th achieved a higher average yield, but not top yield (Fig 72).

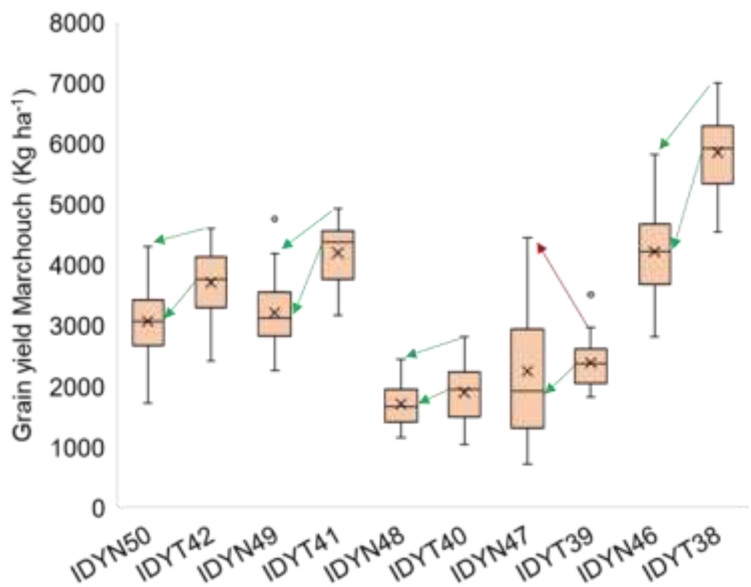


Fig 72. Side-by-side comparison of Stage 4 germplasm from CIMMYT (IDYN) and ICARDA (IDYT) at Marchouch over five seasons

Hence, these results confirm that ICARDA’s program is capable of developing germplasm that is superior to its main competitors (public breeders, private companies, and CGIAR centers) in the target environments. This is evident through the objective appreciation by national partners as demonstrated by the increased requests, a stable genetic gain year after year, and a strong preference for adaptation in Drylands.

Standard operating procedures

This is a list of operations for the technicians to follow that substantially summarize all considerations and plans described in the entire manual.

Crossing block (CB)

- Using seeds from previous CB, Stage 2, 3, or 4, IDSN, IDYN, and academic studies:
    - Prepare one set x 20 g
        - Obregon
    - Prepare two sets x 10 g, two planting dates, one row x 2 m long
        - Terbol or Marchouch
            - Conduct crossing following CB layout
            - Five spikes each

F_1 (off-season)

- Harvest hybrid seeds from CB, bulk seeds of the same cross from five different spikes
    - Put in fridge for 3 days at 4° C to break dormancy
    - Pre-germinate in jiffy pods or cones
    - Transplant in off-season soil under Drip-irrigation
    - Store three seeds in fridge for F_1W
    - Plant in off-season, one row x 2 m long
        - Terbol summer or Annoceur summer

$F_{1:2}$

- Bulk harvest F_1 plants
    - Prepare 50 g for six rows x 3 m long plots
        - Terbol (SR inoculum) or Marchouch (LR inoculation)
- For the F_1 that did not flower, recover remnant seeds in the fridge
    - Pre-germinate in jiffy pods or cones
    - Transplant in one row x 0.5 m long
        - Kfardan (F_1W)

$F_{1:3}$ (off-season)

- Select 40 spikes from $F_{1:2}$ plots (size, flowering time, LR on flag leaf)
    - Bulk-thresh grains
    - Sieve for TKW
    - Put in fridge for 3 days at 4° C to break dormancy
    - Prepare 20 g for planting in two rows x 2 m long
        - Terbol summer or Annoceur summer

$F_{1:4}$

- Bulk harvest $F_{1:3}$ rows
- Obtain seeds from pre-breeding work
- Obtain seed from CIMMYT speed breeding
- Obtain seeds from GS pipeline
    - Prepare two sets of 40 g for six rows x 3 m long plots
        - Terbol

        - Marchouch (apply LR inoculation)
    - Prepare three sets of 5 g for one row x 2 m long
        - Sidi el Aydi
        - Allal tazi
        - Kfardan

F_5 (off-season)

- Select six plants in Marchouch and Terbol and one to two at other sites (spike size, flowering time, LR on flag leaf)
    - Thresh individual plants
    - Put in fridge for 3 days at 4° C to break dormancy
    - Prepare all seeds for planting in one row x 2 m long
        - Terbol summer or Annoceur summer
- For crosses to be developed as mapping populations, randomly select 250 spikes
    - Do not thresh
    - Put in fridge during summer to maintain
- Bulk harvest the plots of $F_{1:4}$ from Marchouch
    - Prepare 50 g for quality testing
        - TKW
        - NIR
        - Yellow pigment
        - Mixograph
    - Prepare 30 g for planting in two rows x 4 m by partner breeding programs:
        - Fanaye, Senegal
        - Amalaha, India
        - Debre Zeit, Ethiopia
        - Others based on request

Stage 1 yield trial ($F_{5:6}$)

- Harvest individual F_5 rows
    - Prepare 40 g for six rows x 3 m long plots
        - Marchouch or Terbol

Pre-IN F_7 (off-season)

- From visually selected plots
    - Harvest two individual plants (short/tall, early/late)
    - Thresh individual plants
    - Put in fridge for 3 days at 4° C to break dormancy
    - Prepare all seeds for planting in one row x 2 m long
        - Terbol summer or Annoceur summer
- Bulk harvest the Stage 1 plots
    - Prepare 50 g for quality testing
        - TKW
        - NIR
        - Yellow pigment

Mixo Stage 2 yield trial ($F_{5:7}$)

- Harvest Stage 1 plot
    - Prepare two sets by two reps of 85 g for six rows x 5 m long plots
        - Marchouch **quality analysis*
        - Terbol
    - Prepare four sets by two reps of 15 g for two rows x 2 m long plots
        - Kfardan
        - Allal Tazi
        - Sidi el Aydi
        - Tessaout
    - Prepare three sets by two reps of 1 g for hill plots
        - Guich under artificial inoculation
        - Guich for seedling screening
        - Guich for Hessian fly testing

Mapping population (F_5)

- Plant directly the stored F_5 spikes in hill plots
        - Marchouch or Terbol
            - Conduct genotyping
            - Harvest $F_{5:6}$ for phenotyping

IN multiplication $F_{7:8}$ (off-season)

- Harvest individual F_7 rows
    - Prepare 40 g for four rows x 7 m long plots
        - AREC
    - Conduct MAS

Stage 3 international nursery IDON ($F_{7:9}$)

- Harvest IN multiplication plots of previous year (staggering)
- Make selection using selection index based on Stage 1 and Stage 2 data, quality (F4, Stage 1 and Stage 2), and MAS
    - Prepare one set of 300 g seeds for four rows x 40 m long plots
        - AREC
    - Prepare four sets of 1,800 seeds for six rows x 5 m long plots
        - Marchouch **quality analysis*
            - *Store 250 g harvest for the short term (60 months)*
        - Terbol
        - Sidi el Aydi **quality analysis*
        - Tessaout **quality analysis*
    - Prepare four sets of 300 seeds for two rows x 2 m long plots
        - Kfardan
        - Allal Tazi
        - Annoceur
        - Melk Zehr
    - Prepare four sets of 1 g for hill plots
        - Guich under artificial inoculation
        - Guich for seedling screening
        - Guich for Hessian fly testing
        - Guich for DNA extraction **genetic passport*

            - *Store seeds in fridge for the medium term (120 months)*
    - Prepare 70 sets of 300 seeds for two rows x 2 m
        - Distribution to requestors

Stage 4 international nursery IDYT ($F_{7:10}$)

- Harvest Stage 3 multiplication plots from AREC
- Make selection using selection index based on Stage 1, Stage 2, and Stage 3 data, quality (F_4, Stage 1, Stage 2, and 3 sites Stage 3), MAS, and GEBV
    - Prepare four sets of 1,800 seeds for six rows x 5 m long plots
        - Marchouch **quality analysis*
        - Terbol
        - Sidi el Aydi **quality analysis*
        - Tessaout **quality analysis*
    - Prepare one set of 1,800 seeds to be split in 15 g for two rows x 2 m long plots
        - Kfardan
        - Allal Tazi
        - Annoceur
        - Melk Zehr
    - Prepare two sets of 1 g for hill plots
        - Guich under artificial inoculation
        - Guich for seedling screening
    - Prepare 70 sets of 1,800 seeds for six rows x 5 m
        - Distribution to requestors

Breeders' seed Stage 4 G_{0-1} F_{11} (off-season)

- Harvest 12 spikes from Stage 4 plot in Marchouch
    - Do not thresh, use spike for hill plots
        - Annoceur summer

Stage 5 national yield trials ($F_{7:11}$)

- Harvest Stage 4 plots from one site (depending on the location)
- Make selection using selection index for the PP of the specific country based on Stage 1, Stage 2, Stage 3, and Stage 4 data, quality (F_4, Stage 1, 2, 3, and 4), MAS, and GEBV
- Include national breeders, private companies, CIMMYT, and ACSAD elites, and major commercial cultivars
    - Prepare sets of two reps 1,800 seeds for six rows x 5 m long plots
        - Plant across locations
            - Conduct quality analysis

Breeders' seed Stage 4 G_2 $F_{11:12}$ (off-season)

- Harvest six homogenous rows from G_1 hill plots in Annoceur off-season
- Thresh individual rows
- Prepare 40 g for six rows x 3 m
        - Marchouch

Stage 5 demo ($F_{7:12}$)

- Harvest Stage 4 plots from one site (depending on the location)

- Make selection using selection index for the PP of the specific country based on Stage 5 only, all germplasm types
- Select:
    - two-four best cultivars
    - two-four lines ready for commercialization
    - two-four new elites
    - Prepare 300-600 g for six rows x 20-40 m long plots
        - Plant at main sites for farmers demonstrations

Breeders' seed Stage 4 G_3 $F_{11:13}$

- Harvest two homogenous plots from G_2 plots
- Thresh plots individually
- Prepare 1 kg for six rows x 100 m
        - Marchouch
            - Harvest 300 spikes from each plot
                - Test one seed per spike with MAS to confirm homogeneity
            - Harvest one strip
                - Store 10 kg of pure seeds for the short term (60 months)

Breeders' seed Stage 4 G_4 $F_{11:14}$

- Harvest one homogenous plot from G_3 strips
- Only for entries that were selected for Stage 5 or release
- Prepare 10 kg for 1,000 m^2 strips
        - Marchouch
            - Harvest 300 spikes from each plot and store temporarily for catalogue submission
            - Store 100 kg and store temporarily for catalogue submission
            - Provide seeds for on-farm and other scaling activities

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