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## The magic of crop wild relatives in durum wheat breeding

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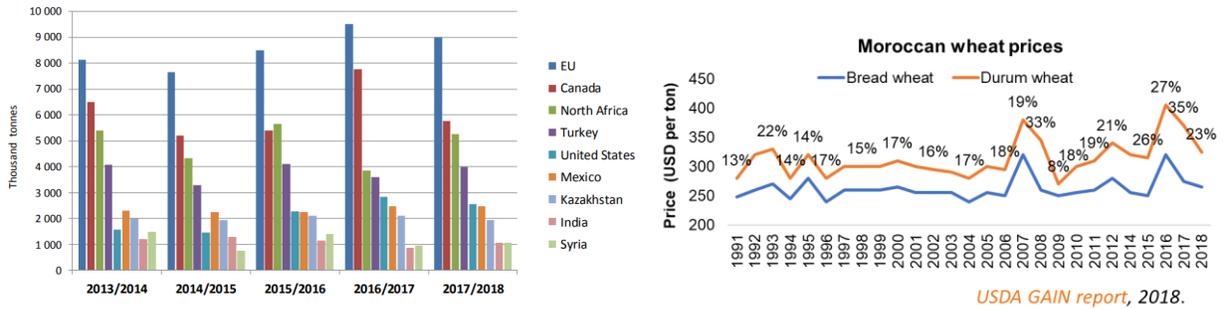
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### Introduction

Durum wheat (*Triticum durum* Desf.,  $2n = 4x = 28$ , AABB) is the tenth most important crop in the world with an estimated production of 40 million tons in 2017 (**Fig 1**). Its global cultivation surface exceeds 18 million hectares, with Canada being the largest cultivator with 2.1 million ha, followed by Italy, Algeria and India with approximately 1.4 million ha each, then Turkey, Morocco, and Syria at 1.0-1.2 million ha, and in order of surface Ethiopia, Tunisia, Iran, France, Spain, Pakistan, and Greece cultivating 0.3-0.5 million ha each. Durum wheat is considered a *staple food* of the Mediterranean diet in the form of unleavened breads, bulgur (concassed grains), pasta, couscous (or Dalhia in India), and its straw is very important as feed for the animals. However, bulgur, pasta, and couscous are now also produced on industrial scale and the market value of the pasta industry alone was estimated at 11 billion USD in 2016, with an expansion trend projected at +20% in the next 5 years. This industry relies on durum wheat to produce high quality semolina. The average price of durum wheat is typically 15-20% higher than bread wheat (**Fig 1**), and premium price of 10-20% over the basic price are paid in developing countries when selling varieties with good grain color (yellow pigment), grain size (hectoliter weight), and especially protein content superior to 13%. In that sense, durum wheat has also become a major *cash crop*, that can provide good returns if the right varieties and agronomic practices are used.

The farming of this tetraploid cereal crop spans a wide a range of climatic zones, varying from warm and dry to cool and wet environments. These are mostly located in areas subject to alternating favorable and stressed conditions depending on annual rainfall and temperatures (Mediterranean-type). Therefore, genetic improvement via breeding for tolerance to biotic and abiotic stresses remains a strategic practice to improve its productivity and stability. In the last decades, many durum wheat varieties have been developed based on field assessment for higher yield, disease resistance and technological seed qualities. However, the strong selection pressure imposed through genetic improvement has eroded a large part of the genetic diversity, hence resulting in a germplasm less prone to adapt to new environmental stresses, diseases and pests. Compared with domesticated varieties, crop wild relatives (CWR) and primitive wheats have been challenged in natural environments for thousands of years and maintain a much higher level of diversity. Hence, interspecific hybridization between durum elite lines and wild relatives of

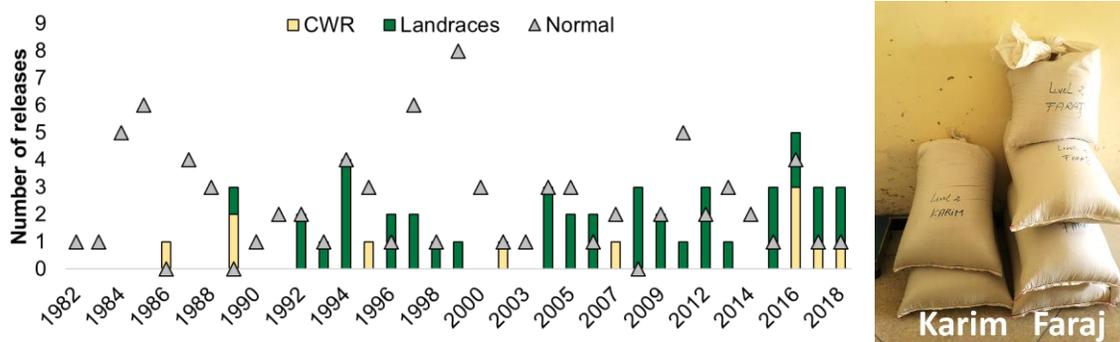
the Gramineae family is a promising method to restore variability to the modern breeding germplasm.



**Fig 1.** Global producers of durum wheat (adapted from International Grain Council report 2018) and Moroccan annual average prices for durum and bread wheat (adapted from USDA GAIN report 2018).

### ***Breeding varieties via Crop Wild Relatives (CWR)***

Several useful traits have been identified in species related to durum wheat. Regardless of the proven usefulness of CWR for trait discovery and deployment in pre-breeding, wheat breeders have often regarded this type of crosses as a “last resource” that will inevitably require several years and multiple recurrent cycles before delivering a promising candidate for variety release. This vision is mostly associated with the risk indicated by several authors that undesirable linkage drags exist between useful wild traits and negative yield or quality alleles. One of the exceptions to this breeding trend has been the ICARDA durum wheat program that broadly utilized CWR and landraces into their hybridization strategy (Nachit and Elouafi., 2004). ICARDA’s gene bank collection is among the largest in the world with over 43,000 accessions of wheat, mostly composed of landraces and CWR. This treasure trove has been extensively utilized by ICARDA’s breeders to generate huge impact in farmers’ fields, with a special focus to those dry areas where the harsh conditions made it impossible for other breeding strategies to work effectively. The durum wheat germplasm supplied by ICARDA has allowed national programs in 22 countries to release 125 varieties, of which 8% were derived from CWR, and 30% by landraces (**Fig 2**). Among the 10 most recent varieties released in 2016 and 2017, 4 were derived from top-crosses with CWR and 2 from landraces. Morocco released in 2017 the variety ‘Nachit’ (Amedakul 1/*Triticum dicoccoides* Syrian collection//Loukos) extremely well adapted to the dry conditions of the high-plateaus thanks to its deep root system and large grains. On-farm and on-station testing on 0.1 ha plots of this new variety revealed a significant 25% yield advantage over the most grown variety ‘Karim’. But Morocco is not new of releasing ICARDA’s wide crosses. Already in 2007 a variety under the name ‘Faraj’ was derived from crosses with *Triticum araraticum*. This variety was specifically released to target the driest areas of Morocco, where Hessian fly is a major issue. Testing of ‘Faraj’ on-station and on-farm revealed a clear yield advantage under dry conditions (**Fig 2**).



**Fig 2.** Origin of 125 durum wheat varieties released from ICARDA’s germplasm, and comparative performances of the most grown Moroccan cultivar ‘Karim’ against the 2007 release from CWR ‘Faraj’.

In Mauritania and Senegal, two varieties were released in 2017 ‘Amina’ (Korifla/*Ae. speltoides*//Loukos) and ‘Haby’ (Mrb5/*T. dicoccoides* Aleppo Col//Cham1) also derived from simple top-crosses with *A. speltoides* and wild emmer, respectively. These new varieties were selected under relentless high temperatures for a period of four years, to reveal the ability of maintaining high spike fertility under heat stress. Likely this trait is derived from their more primitive genetic makeup. The release of durum wheat varieties for the Senegal River region was recognized as a major achievement and awarded the 2017 OLAM Prize for Innovation in Food Security (<http://olamgroup.com/sustainability/food-prize-re-imagine-agriculture/>).

### ***CWR for pre-breeding efforts***

The direct use of CWR as parental lines for the ICARDA’s breeding program has resulted in a genetic diverse germplasm, capable of adapting to extreme agro-ecological stresses. However, each new cultivar includes only some combination of positive wild and elite alleles, but not all. Hence, there is scope to attempt to pyramid the most useful alleles from different sources into a superior cultivar. The first step in that sense is to define the specific traits and genomic regions involved. To do so, a panel including ICARDA’s CWR-derived elites as well as germplasm from around the world was assembled and genotyped with 35K Axiom array. This set of germplasm was tested under different conditions to reveal QTLs and traits of interest. The studies conducted, and outcomes are summarized below:

1. *Genetic diversity*: a study by Kabbaj et al. (2017) revealed the existence of 6 sub-populations among the modern germplasm, with ICARDA’s being part of 4 of them. That study also revealed that the CG programs have been able to capture up to 21% of the variation for rare alleles (CIMMYT), and 51% of the diversity for common alleles (ICARDA), which is significantly lower than what observed for Central and South Asia landraces, which reached 62% and 84%, respectively, but superior to other breeding programs.
2. *Hessian fly resistance*: a study by Bassi et al. (2019) revealed the source of Hessian fly resistance from *Triticum araraticum* to be located in the telomeric portion of chromosome 6BS in the progenies derived from the ‘Faraj’ cultivar.
3. *Heat tolerance along the Senegal River*: a study by Sall et al. (2018a, b) identified that the capacity of maintaining high fertility (grain number per spike) under severe heat was the key to ensure tolerance to constant daily temperatures above 34°C. Further, it was possible to identify seven genomic regions linked to this trait, with one QTL on chromosome 4AS and

originated from *A. speltoides* accounting for an average yield advantage 669 Kg ha<sup>-1</sup> under severe heat.

4. *Heat tolerance under plastic tunnels*: a study by Hassouni et al. (unpublished) revealed that applying a plastic tunnel at the time of flowering, raises the daily temperatures to up to 46° C and causes a yield reduction of up to 60%. As per the study by Sall et al. (2018a, b), the key trait for tolerance was the maintenance of spike fertility. Further, this study was able to identify 6 genomic regions controlling heat tolerance, 3 of which overlap with the study along the Senegal River.
5. *Drought tolerance via root architecture*: a study by Hassouni et al. (2018, unpublished) revealed that mature and seminal root angles are controlled genetically and a narrower root angle (deeper roots) can ensure up to 39% yield advantage under terminal drought conditions. Genomic scan revealed three regions controlling this trait with additive effects, but just one major locus on chromosome 7B, originated from wild emmer wheat, ensures up to 500 Kg ha<sup>-1</sup> yield advantage when terminal drought occurs.
6. *Drought tolerance via yield testing*: a study by Zaim et al. (2017, unpublished) conducted yield trials across 21 drought-prone and favourable sites to reveal that grain size is the most critical yield component for tolerance against terminal drought, together with high biomass. The combination of G and GxE components (Bassi and Sanchez-Garcia, 2017) has allowed to identify CWR-derived elites as the best performing ones, thanks to four major QTLS on chromosomes 2B, 5A, 5B, and 7B.
7. *Industrial quality across 5 sites*: a study by Azouz et al. (unpublished) used the harvest across 5 sites (2 drought-prone, 1 flood irrigated, and 2 with fertigation) to measure the milling and transformation property. It was possible to confirm that CWR-derived lines had significantly larger grain size, better semolina yield, higher protein content, superior virtuousness and yellow pigment. Gluten strength was instead superior in germplasm coming from Canada and Australia. The line ‘Syriopsis’, derived from *A. speltoides*, was identified as accumulating the highest rate of selenium in the grains, an important micronutrient that can be used to raise market value.
8. *Conversion and validation for MAS*: over 120 marker-trait associations for the different stresses described above were converted to KASP markers and used for validation on an independent germplasm set. A total of 20 markers explained more than 10% of the phenotypic variation targeted and can now be used for MAS (**Table 1**).

### **Conclusions**

Several field and artificial assessments for different traits and different stresses have been completed. The results are extremely clear: CWR-derived elites are superior to elite-by-elite lines. Their superiority is mainly linked to higher spike fertility under extreme heat, deeper root system under drought, larger grain size, and several added-value characteristics. In addition, 20 KASP markers have been validated and linked to these traits, making pyramiding easier. Hence, the results presented here suggest that the only true limitation that shall prevent durum wheat breeders from taking full advantage of CWR would be their unwillingness to do so. For that reason, ICARDA, its national partners in Morocco, Lebanon, Senegal, and Ethiopia, and the Crop Trust have partnered with funding from NORAD (Norway) to focus on the delivery to farmers fields of these superior CWR-derived cultivars, with a special focus to those regions that suffer from the harshest drought and heat damages.

**Table 1** Validated KASP from association mapping studies

<b>KASP code</b>	<b>QTL</b>	<b>Scope</b>	<b>Chr</b>
BS00009709	QTL.ICD.RL3.001	Drought tol	1A
AX-94733268	QTL.ICD.RL2.001	Drought tol	1B
AX-95176186	QTL.ICD.RA.006	Drought tol	3B
BS00022364	QTL.ICD.GYKFD17A.001	Drought tol	4B
AX-94383178	QTL.ICD.RA.011	Drought tol	6B
AX-94549122	QTL.ICD.HSI-GY.002	Heat tol	1B
AX-94509297	QTL.ICD.HSI-TKW.002	Heat tol	1B
BS00004224	QTL.ICD.PlstTun.TKW.001	Heat tol	2B
AX-94681091	QTL.ICD.PlstTun.GY.003	Heat tol	3A
AX-94932858	QTL.ICD.HSI-GY.003	Heat tol	3B
AX-94679648	QTL.ICD.GYheat.004	Heat tol	4B
AX-95260810	QTL.ICD.PlstTun.GY.006	Heat tol	5A
AX-94432276	QTL.ICD.PlstTun.GY.006	Heat tol	5A
AX-95182463	QTL.ICD.PlstTun.GY.007	Heat tol	5B
AX-94622179	QTL.ICD.HSI-GY.007	Heat tol	7B
BS00072387	QTL.ICD.Hara.6B	Hessian fly res.	6B
AX-94385320	QTL.ICD.Ppd.002	Phenology	2A
AX-95213349	QTL.ICD.Vrn.006	Phenology	5A
AX-95140644	QTL.ICD.Vrn.007	Phenology	5B
AX-95115092	QTL.ICD.AWAI.007	Stability	4B

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