





SPECIAL ISSUE ARTICLE

Adapting Agriculture to Climate Change: A Walk on the Wild Side

Crop wild relatives in durum wheat breeding: Drift or thrift?

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Abstract

Crop wild relatives (CWRs) are an important source of genetic diversity for crop improvement. The aim of this study was to assess the usefulness of deploying CWRs in durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) van Slageren] breeding. A set of 60 accessions was selected to include cultivars from nine countries, top lines obtained via elite-by-elite crossing, and CWR-derived lines. These accessions were screened for resistance against four major fungal diseases to reveal that CWR-derived lines are a good source of resistance against Septoria leaf blotch (*Zymoseptoria tritici*), while they were highly susceptible to tan spot (*Pyrenophora tritici-repentis*). Drought tolerance was assessed at eight environments with contrasting nitrogen levels and tillage practices to reveal a clear superiority of CWR-derived lines for grain size as well as higher grain yield (GY) under low nitrogen and normal tillage (NT). Temperature-stress tolerance was assessed at four heat-stressed environments along the Senegal River to confirm CWR-derived had up to 42% yield advantage and a higher grain number per spike (GNspk). Combined testing under plastic heat tunnels imposed at the time of flowering also revealed good performance of CWR-derived lines. However, the CWR-derived lines had low gluten sedimentation index and poor yellow color compared with cultivars and elite germplasm. High genetic diversity was found in CWR-derived lines with 75% of individuals having minor allele frequency (MAF) of 40–44% for frequent alleles but low genetic diversity for alleles with low frequency. In addition, 8–13% of the CWR parent genome

Abbreviations: CWR, crop wild relative; GNspk, grain number per spike; GY, grain yield; MAF, minor allele frequency; MR, moderately resistant; MS, moderately susceptible; NT, normal soil tillage; SedInd, sedimentation index; SSA, sub-Saharan Africa; TKW, 1,000-kernel weight; YP, yellow pigment; ZT, zero tillage.

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was retained in the derived progenies, which contributed to improve several phenotypic traits.

1 | INTRODUCTION

Durum wheat history begins some 12,000 yr ago in the Fertile Crescent where a wild progenitor, wild emmer [*T. turgidum* L. subsp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell.], was domesticated by farmers to cultivated emmer [*T. turgidum* L. subsp. *dicoccon* (Schrank) Thell.] (Gioia et al., 2015; Ren et al., 2013; Zohary, Hopf, & Weiss, 2012). The origin of the wild emmer is still controversial, but the overall consensus is that the durum genome (AABB) resulted from a natural hybridization between diploid wild einkorn (*T. urartu* Tumanian ex Gandilyan) (AA genome) (Monneveux, Zaharieva, & Rekika, 2000) and an unknown diploid *Aegilops* species (BB genome), most probably related to *A. speltoides* Tausch (Biswas et al., 2008; Haudry et al., 2007; Valkoun, 2001). The further domestication by farmers of cultivated emmer resulted in what is today known as durum wheat. Its actual place of origin is also somewhat controversial, but recent molecular data support a double origin: first in the southern Levantine and later in Ethiopia (Civán, Ivaničová, & Brown, 2013; Kabbaj et al., 2017; Takenaka, Mori, & Kawahara, 2010).

Durum wheat cultivation expanded during human civilization until becoming one of the predominant crops worldwide to then be quickly replaced by common wheat (*T. aestivum* L.) in recent times. Today, durum is the second most widely cultivated wheat crop and contributes to the global production with 34 million t harvested in 2019 (Arjona et al., 2020; Cooper, 2015; European Commission, 2020). Its area of cultivation is concentrated principally in the Mediterranean area, eastern Africa, North American Great Plains, India, and western and central Asia (Mengistu & Pe, 2016; Sall et al., 2019). Durum wheat holds great importance as raw material for the preparation of traditional and industrial foods such as pasta, bulgur, couscous, and unleavened breads (Alsaleh, Baloch, Nachit, & Özkan, 2016). In sub-Saharan Africa (SSA), Ethiopia is the biggest producer with ~0.6 million ha and the only country in SSA able to produce pasta from locally grown grains (Biggeri, Burchi, Ciani, & Herrmann, 2018; Sall et al., 2019).

Durum grains typically receive higher market prices when their protein content exceeds 13%, as this is a key requirement for industrial transformation (Luo et al., 2018). For this reason, farmers tend to cultivate durum in marginal lands or planted later in the season to favor high protein concentration. This practice exposes the crop to several environmental stresses such as terminal moisture stress or high temperatures during the reproduction phase (Li, Wu, Hernandez-Espinosa, & Peña, 2013; Soriano, Villegas, Sorrells, & Royo, 2018) and to biotic stresses such as Hessian fly [*Mayetiola destructor* (Say)] (Bassi et al., 2019; Nsarellah, Amamou, Taghouti, & Annicchiarico, 2011), rusts (De Vita & Taranto, 2019; Miedaner, Rao, Flath, Longin, & Wurschum, 2019; Terracciano et al., 2013), and blotches (Faris et al., 2020; Ouaja et al., 2020). Breeding of new cultivars well adapted to these stresses is key to ensure an increase in productivity and profitability of this crop as well as having a positive impact on the natural environments (Brummer et al., 2011). Crop wild relatives are species related to modern crops including their ancestors. Because CWRs have not undergone the process of human selection and domestication but rather have survived against various challenges in their natural habitats and are deemed to possess a vast array of useful alleles and to be a source for increasing genetic diversity (Hodgkin, Hajjar, & Maxted, 2008; Jarvis, Lane, & Hijmans, 2008; Maxted & Kell, 2009; Vollbrecht & Simon, 2005). In durum wheat, several research undertakings have proven the advantage of using CWR hybridization to derive superior cultivars. For instance, wild emmer germplasm was shown to harbor important beneficial alleles to improve disease resistance (Maccaferri et al., 2019), nutritional quality (Çakmak et al., 2004), and drought tolerance (Reynolds, Dreccer, & Trethowan, 2007). Bassi et al. (2019) confirmed that a major source of resistance against Hessian fly originated from a translocation of *T. araraticum* Jakubz. to the telomeric portion of chromosome 6BS. Zaïm et al. (2017) tested the possible negative effects of reintroducing primitive diversity by comparing the performances of three ICARDA elites and four

commercial cultivars against 17 durum wheat wide crosses generated by hybridization to wild emmer, *T. araraticum*, and *A. speltooides*. The results revealed that wide crosses had significantly higher levels of resistance to diseases, better yield potential and stability, larger grain size and higher protein content but suffered of poorer end-use quality. Sall et al. (2018a,b) tested CWR-derived lines under severe heat stress along the Senegal River to confirm their yield superiority. In particular, the capacity of CWR-derived lines of maintaining high fertility (GNspk) under severe heat was the key to tolerate constant daily temperatures above 34 °C. A further study on heat tolerance by El Hassouni et al. (2019) identified a CWR-derived line among the top five entries evaluated under severe heat by applying a plastic tunnel at the time of flowering, which raises the maximum temperatures to up to 46 °C. Talini et al. (2019) reported superior end-use quality alleles carried by wild einkorn for glutenins, carotenoid concentration, and sedimentation volumes. Furthermore, it is anticipated that the integration of CWRs into breeding will become increasingly common thanks to the recent advances in molecular technologies that ensure better efficiency and accuracy in transferring desired traits from CWR to crops (Hajjar & Hodgkin, 2007; Prohens et al., 2017).

The superiority of CWR-derived lines for specific traits has been presented by many but often in unbalanced sets using only limited trials or focusing on CWRs themselves without achieving the transfer to modern germplasm. Here, we aim to assess the potential of using CWR-derived top lines for durum wheat breeding against a balanced set of cultivars and elites across several environments and for multiple traits: disease resistance, drought tolerance, heat tolerance, and end-use quality. Further, we extended the study to the molecular level to determine what fraction of the CWR genome is really inherited by their progenies under breeding selection and how much genetic diversity it contributes.

2 | MATERIAL AND METHODS

2.1 | Germplasm used

A large panel comprising 92 landraces and 292 modern lines of durum wheat was tested for various traits. Full details are described in Kabbaj et al. (2017). In this article, we referred to the full set of germplasm as ‘whole panel.’ The definition ‘panel’ was instead assigned to 144 entries used to conduct field assessment for drought tolerance across eight sites. These entries were selected from the initial whole panel to be well-representative of its genetic diversity while allowing replicated field trials at reasonable

costs. A third set of 42 lines was used for assessing heat tolerance within plastic tunnels as described in El Hassouni et al. (2019) to account for the hard labor and costs of this type of assessments. For simplicity, this is also referred to as ‘panel’ here. The word ‘subset’ is specifically used to define only the 60 accessions described in this work, which included 20 cultivars obtained from nine countries (Australia, Canada, Italy, Lebanon, Mauritania, Morocco, Senegal, Spain, and United States), 20 elite lines developed by the ICARDA’s breeding program in 2015, and 20 additional elites developed in the same period but which incorporated CWR in their pedigree. Among this set of CWR-derived lines, nine were derived from top-crosses (A/CWR//B) with one Syrian biotype of *A. speltooides*, three from top-crosses with a Syrian biotype of wild emmer, and nine from complex crosses involving *T. araraticum* obtained from Kansas State University as a source of resistance to Hessian fly (Bassi et al., 2019). These three contrasting germplasm types (cultivar, CWR-derived, and elite) were assessed for various phenotypic and genotypic aspects to determine their usefulness for breeding. In addition, the five parents used for making the CWR top-crosses were included only for the genotyping part of this work (Heider, Om Rabi 5 syn. Cham 5, Amedakull, Korifla syn. Cham 3, and Waha syn. Cham 1). Full details of the germplasm can be found in Supplemental Table S1.

2.2 | Phenotyping

Four major traits were used to characterize this set of germplasm: disease resistance, drought tolerance, heat tolerance, and end-use quality. For each one of these traits, the frequency of good and bad accessions was derived for each germplasm type under study in an attempt to determine for which traits the use of CWR-derived proved advantageous over cultivars and elites. The description of each phenotypic evaluation follows and the ratio of germplasm tested for each is reported in Table 1.

2.2.1 | Fungal disease screening

The reaction to four major damaging leaf diseases was assessed: leaf rust (*Puccinia triticina*), yellow rust (*P. striiformis* f.sp. *tritici*), septoria leaf blotch (*Zymoseptoria tritici*), and tan spot (*Pyrenophora tritici-repentis*). For the two rusts, four seasons of field evaluations under natural infection were conducted at the Moroccan site of Allal Tazi using spreader rows of the susceptible cultivars Waha and Om Rabi 5. Races were annually tested by the University of Minnesota to confirm that the predominant complex of

TABLE 1 Germplasm characterization for different traits and trials expressed as fraction of total entries

Type	N	Ratio tested				
		Diseases	Drought	Heat	Quality	Genotyping
		%				
Cultivar	20	100	100	30	100	100
Crop wild relative-derived	20	100	85	35	100	100
Elite	20	100	95	75	100	100
Overall	60	100	93	47	100	100

leaf rust races were BBBS, BBBQ, MCDS, and MCDF with combined virulence on Lr: 1, 3, 3bg, 10, 14a, 17, 26, and B (Long & Kolmer, 1989) and for yellow rust was PstS13 and PstS14 with combined virulence on Yr: 2, 3, 6, 7, 8, 9, 17, 25, 32, Sp, and AvS (Hovmoller, Rodriguez-Algaba, Thach, & Sorensen, 2017). For septoria leaf blotch, mist irrigation was deployed after two spray inoculations, and one straw inoculation of a mixture of local isolates over two seasons, at the station of Guich in Rabat (Morocco). Tan spot was screened at three sites over two seasons following the indications presented by Gamba, Bassi, and Maria (2017) to challenge the germplasm with local most prevalent races 1, 5, 6, and 7. The experimental layout of the whole panel for all field experiment was an augmented design with 19 blocks each including the same four checks: the ICARDA's cultivar Waha (syn. Cham 1), Om Rabi 5 (syn. Cham 5), Azeghar 2 (syn. Lahn3), and Icarasha 2 (syn. Mukiye). The rusts scores were converted to the coefficients of infection by multiplying the virulence score by the disease spread (Zaïm et al., 2017). These continued values were converted to BLUP for each individual environment using the `Dau.test` function of the R package *agricolae* (de Mendiburu, 2012; de Mendiburu & Simon, 2015). The score of each individual environment was combined across sites and year for each disease type, assuming environment as random and genotypes as fixed in a linear model using the `lme4` package in R v 3.4.3 (R Core Team, 2017). The least significant difference (LSD) was calculated for the combined analysis by the `LSD.test` function of *agricolae* (de Mendiburu, 2015; R Core Team, 2017) and used to define four classes of disease response for each genotype: resistant for values between 0 and the LSD, moderately resistant (MR) for values up to twice the LSD, moderately susceptible (MS) up to three times the LSD, and susceptible. The disease response score of the subset was then used to determine how many of the 20 accessions of each type of germplasm tested here (cultivar, CWR-derived, and modern) belonged to each disease response class. In addition, it was also measured how many accessions were simultaneously resistant or MR to both rusts, both blotches, and all four diseases. To statistically test the difference in dis-

ease response frequencies of the three germplasm types, ANOVA was performed using the germplasm types as fixed effect and the response of the individuals within types as random. The LSD values were derived for each single disease and combination of diseases.

2.2.2 | Terminal drought stress test

Grain yield measured in occurrence of drought is ultimately the best measure to determine the ability of a plant to adapt to the stress. The 1,000-kernel weight (TKW) instead has been identified as one of the most critical traits for drought tolerance (Mohammadi, Karimizadeh, Shefazadeh, & Sadeghzadeh, 2011). A total of eight environments experiencing severe terminal drought were used to assess the whole panel for GY and grain size (TKW). The Moroccan site of Marchouch was planted in season 2016–2017 and 2017–2018 using two seeding method: sowing after NT and direct sowing after zero tillage (ZT). The same seeding machine and management practices were used for both treatments, the only difference was two soil tillages by cover crop. The experimental station of Marchouch received a total rainfall of 280 and 390 mm during 2016–2017 and 2017–2018 seasons, respectively, with no rain falling after the day of flowering. The station of Kfardan in Lebanon was also used in seasons 2016–2017 and 2017–2018 to assess terminal drought stress tolerance. Two treatments were also imposed at this station: a basal nitrogen (N) application for both treatments equivalent to 20 kg ha⁻¹, followed by 40 kg ha⁻¹, which is the counselled dose for this environment (treatment normal N), or just 10 kg ha⁻¹, which represents severe N deprivation (treatment low N). At both sites a panel of 144 accessions selected from the whole panel was planted in plot sizes of 7 m² following an alpha lattice design with two replicates and 12 incomplete blocks of size 12. The META-R software (Alvarado et al., 2017) was used to calculate the best linear unbiased predictions of each individual environment and the relatedness between the sites (Supplemental Figure S1). The actual values were then converted to a ratio of

the mean of the trial and averaged across the two seasons and also across all environments. In total, five field scores for each of the two traits (GY and TKW) were obtained: combined across all eight environments, low N, normal N, NT, and ZT. These field scores were recorded for 56 of the 60 accessions under study. Each germplasm type was then defined as fixed and the genotypes as random to run a mixed linear model for each trait individually. The LSD was defined to compare the average response of each germplasm type.

2.2.3 | Heat stress screening

Grain yield measured in occurrence of heat at the time of flowering is deemed the best measure to assess a plant capacity to adapt to the stress (Kamrani, Hoseini, & Ebadollahi, 2018), while the GNspk has been identified as one of the main traits for tolerance (El Hassouni et al., 2019; Sall et al., 2018a,b). The whole panel was evaluated over two seasons at two heat-prone field stations in Kaedi, Mauritania and Fanaye, Senegal with maximum daily temperatures exceeding 34 °C throughout the two seasons for a total of four environments (2 seasons × 2 sites). Full details of this experiment are presented in Sall et al. (2018a,b). The heat tolerance of the genotypes was determined by presenting GY and the GNspk as ratio to the average across sites. A panel of 42 accessions were also evaluated under normal and induced heat-stress conditions at the station of Marchouch in Morocco over two seasons as described in El Hassouni et al. (2019). Briefly, the terminal heat stress was generated by covering the plots with plastic tunnels at the time of flowering for a period of 15 d, which raised the temperatures by up to +16 °C. For both heat stress tests, GY and GNspk were recorded, and the experimental designs were resolved to derive best linear unbiased estimates for both traits. For the field trials along the Senegal River, the values were expressed as ratio to the average performance. For the plastic tunnel, a heat tolerance index (HTI) was calculated as explained in El Hassouni et al. (2019):

$$HTI = \frac{(Y_s/Y_p)}{(\bar{Y}_s/\bar{Y}_p)}$$

where Y_s and Y_p are the trait value measured under stressed and normal conditions, respectively, while both \bar{Y}_s and \bar{Y}_p are the mean trait value of the population under study under stressed and normal conditions, respectively. To define genotypes with good resistance to heat, the HTI was calculated for GY and GNspk as ratio to the average of the two seasons, with accessions performing better than the average deemed as tolerant.

2.2.4 | End-use quality screening

The panel was grown at Marchouch in season 2016–2017 and 2017–2018 under NT and ZT methods as described for drought screening. The seeds obtained from the trials were cleaned, normalized at 13% moisture, and used for end-use quality screening at the laboratory of ICARDA, Morocco. Protein content was determined using a Chopin Technologies Infraneo near-infrared spectroscopy. The grains were then processed using a grinder (Udy-Cyclone 0.5 MMM sieve) to obtain whole-meal flour. A chroma meter Konica Minolta (CR-400) was used to determine the yellow pigment (YP) as the score of b^* . Sodium dodecyl sulfate digestion was used to determine the levels of gluten strength of each sample following a Moroccan standard method (N.M.08.1.217, 1999) equivalent to American Association for Cereal Chemistry method (AACC 56–70). A sedimentation index (SedInd) was then calculated by multiplying the protein content by the gluten strength value. The YP and SedInd were statistically resolved in BLUP for each environment using the experimental design of the whole panel as explained before. The actual values were then converted to a ratio of the mean of the trial. To define the best accessions, the environments were used a random effect and LSD calculated. To then test the differences among germplasm types, these were considered as fixed effects and the genotypes as random to determine LSD differences for the average of the class combined across environments.

2.3 | Genotyping

The 35K SNP Axiom breeders' array was used as described in Kabbaj et al. (2017) for genotyping. Because of the scope of this study, a different markers curation was adopted compared with what was described in Kabbaj et al. (2017). A total of 22,589 SNPs were retained by also incorporating markers with MAF of just 2% in an attempt to capture diversity for rare alleles.

2.3.1 | Shifts in minor allele frequencies

Five markers classes were then defined based on the MAF calculated across the whole panel: frequent alleles (49–30%), common alleles (30%–20%), rare alleles (20%–5%), and unique alleles (5%–2%). In total, 5,389; 2,764; 7,940; and 6,505 markers were identified as belonging to the different classes, respectively. The average MAF of each genotype was then measured for each marker class. Using this average value, the genotypes were then assigned to four MAF classes corresponding to one-sixth increments. The

distribution was then displayed for the whole panel and the three germplasm types. To determine if the distribution of any point of the curve was significantly different than the average of the whole panel, the germplasm types and the six MAF classes were defined as fixed and the individuals within each germplasm type as random (i.e. replicates). A linear model was then run to derive the LSD using R package agricolae (de Mendiburu & Simon, 2015).

2.3.2 | Genomic contribution of crop wild relatives in top crosses

Ten of the CWR-derived genotypes were obtained by top crossing CWRs to two elite parents (P1/CWR//P2). In order to define the genomic contribution of the two parental lines these were also genotyped by Axiom 35K array. A direct comparison was done at all loci to define if the allele carried by the CWR-derived line originated by one or the other parent or both (i.e., monomorphic). Any allele present in the CWR-derived line but absent from both parents was deemed to have originated from the CWR used in the cross. The four possibilities (P1, P2, both parents, or CWR) were then expressed as percentage of alleles overall. To further test if the CWR-derived lines had higher genetic diversity than the two parents, a MAF comparison was carried out by subtracting the highest MAF score of P1 or P2 from the MAF value calculated for the CWR-derived line considering the four marker classes defined before.

3 | RESULTS

3.1 | Disease response of different germplasm classes

Four major durum wheat diseases were considered: leaf rust, yellow rust, septoria blotch, and tan spot. The average across four environments was used to determine the disease response class (resistant, MR, MS, susceptible) of the 60 lines tested here (Figure 1; Supplemental Table S1). The ANOVA test for four response classes assigned to the germplasm types revealed significant differences for all four diseases (Supplemental Table S2). For leaf rust, the elite set had significant ($p < .01$) higher frequency of resistant genotypes (55%) than the CWR-derived set (25%), while comparison for the other disease response classes was not significant. For yellow rust, no significant differences were observed between germplasm types. In the case of septoria blotch, the CWR-derived set had a significantly higher rate (30%) of genotypes with moderately resistance response vs. the other two germplasm types (10%). Finally, for tan spot, the CWR-derived set had significantly lower

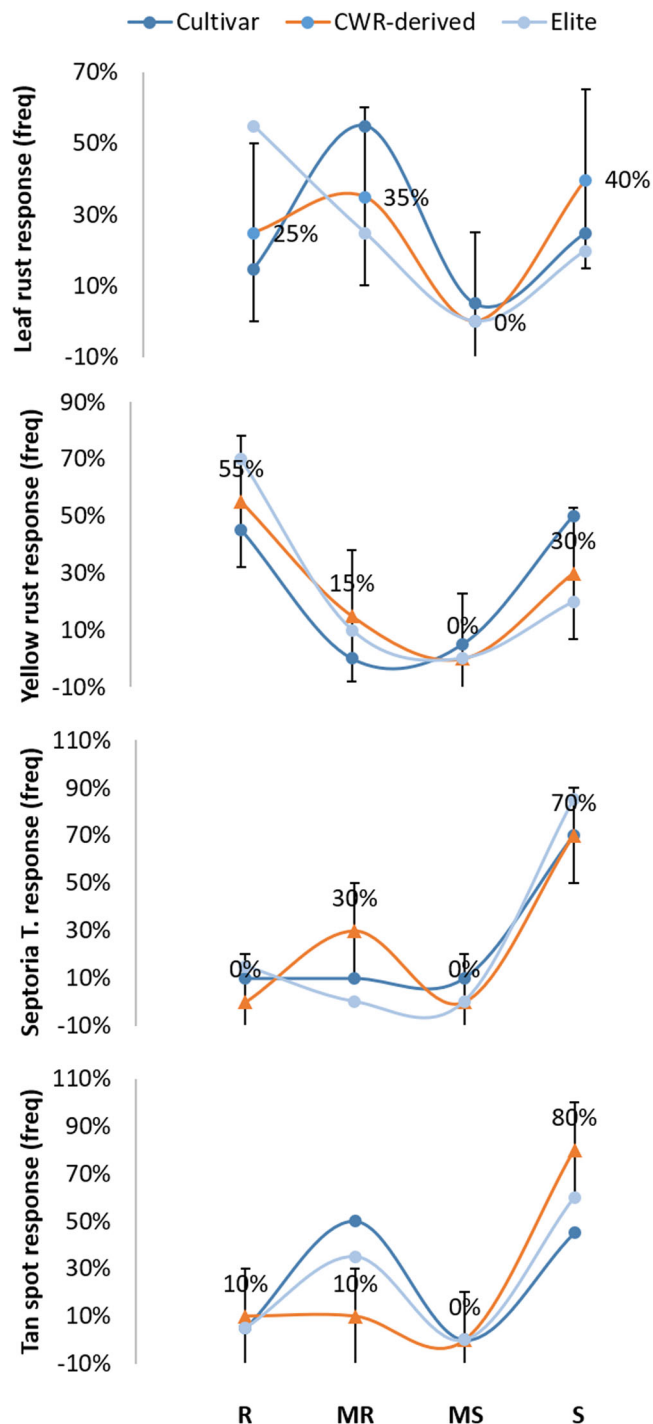


FIGURE 1 Frequency distribution of three germplasm types (cultivar, crop wild relative (CWR)-derived, and elite) into disease response classes (R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible) for four major fungal diseases. The error bars on the graph show the LSD value plotted on the CWR-derived line. The numerical value represents the score recorded for CWR-derived lines

TABLE 2 Frequency distribution of combined disease responses (resistant [R], moderately resistant [MR], moderately susceptible [MS], and susceptible [S]) of three germplasm types against leaf and yellow rusts, septoria and tan spot blotches, and all four diseases

Germplasm types	Combined two rusts		Combined two blotches		Combined all diseases	
	R-MR	S-MS	R-MR	S-MS	R-MR	S-MS
	%					
Cultivar	35b ^a	20a	10a	35a	5a	5a
Crop wild relative-derived	40b	10a	5a	55a	5a	5a
Elite	70a	10a	10a	55a	10a	0a
LSD	27		26		14	

^aWithin columns, LSD means followed by the same letter are not significantly different at $p < .01$.

frequency of MR genotypes (10 vs. 35%) and a higher frequency of susceptible accessions (80 vs. 60%).

The analysis of germplasm distribution for combined resistances (Table 2) identified a significant difference among germplasm types, with the elite set having 70% of the tested accessions reaching resistant or MR response against only 40% of the CWR-derived lines. No significant differences in frequency distribution could be identified for the combined blotches and all four diseases. Conversely, the elite set recorded 10% of the tested germplasm as resistant or MR against all four diseases, while for cultivars and CWR-derived, only 5% of the tested lines reached this score.

3.2 | Drought tolerance response of different germplasm classes

A total of two sites, each with two different treatments and two seasons were used to evaluate the drought tolerance of the three germplasm types. The ANOVA for the whole panel revealed significant ($p < .01$) genotype effect at each site (Supplemental Table S3). The ANOVA test of differences between germplasm types (Supplemental Table S4) indicated significant type differences ($p < .1$) for two environments and combined analysis for TKW and for three environments for GY. The combined value of the germplasm types across seasons for each treatment and combined across treatments is presented in Figure 2 as the ratio to the trial mean and in Supplemental Table S1 as actual value. For TKW, the CWR-derived set showed significant superiority to the other two germplasm types under NT and ZT and when considering all environments combined. The nitrogen trials instead did not result in significant differences among germplasm types even though larger average scores were also recorded for the CWR-derived set. The combined analysis for GY did not reveal any significant germplasm superiority, while under low nitrogen the CWR-derived lines outyielded the other two germplasm types but under normal nitrogen the cultivar set excelled. Under NT the CWR-

derived set outperformed the cultivars, while no significant difference could be identified for ZT. Overall, the CWR-derived lines showed significant larger grain size (TKW) under drought and superior GY for two of the four treatments.

Among the genotypes assessed, the following entries reached the highest TKW (43 g) in the combined analysis Cvr-10 (Isly, from Morocco), Elite-01, *A. speltooides*-derived (Wide-03, Wide-06, Wide-09), Wide-15 (derived from *T. araraticum*), and Wide-20 (derived from wild emmer and corresponding to the 2018 released Moroccan cultivar Nachit). Interestingly, Wide-20 (Nachit) resulted as the top TKW for ZT and low N at 54 and 41 g, respectively, two management practices of great interest for the promotion of sustainable intensification. For GY, the combined analysis identified as top yielding with over 2,790 kg ha⁻¹ Cvr-18 (Saintly from Australia), Elite-07 (CIMMYT), Elite-17 (Kunmiki), Wide-03 (derived from *A. speltooides*), and Wide-20 (Nachit). Under ZT, the Australian cultivar Saintly was the overall top yielder, together with Wide-03, while under low N the best two were CWR-derived Wide-18 (Jabal from *A. speltooides*) and Wide-03 also from *A. speltooides*. The Moroccan cultivar Nachit resulted among the top six for both ZT and low N.

3.3 | Heat stress tolerance

Four field environments under continuous heat stress along the Senegal River were combined with four artificial environments where heat stress was imposed at the time of flowering. The response of individuals was averaged across the two heat-tolerance tests and presented as ratio to the average of the trial for GY and GNspk in Figure 3 and in Supplemental Table S1 as actual value. With regards to GY, 42, 42, and 50% of the tested individuals resulted superior to the average in both experiments (top right corner of Figure 3) for CWR-derived, cultivars, and elite germplasm types, respectively. The top yielding lines along the Senegal River were Cvr-03 (Sudanese cultivar Berghouata1), Elite-13 (IDON37-062), Elite-19 (Moulsabil2), and Wide-16

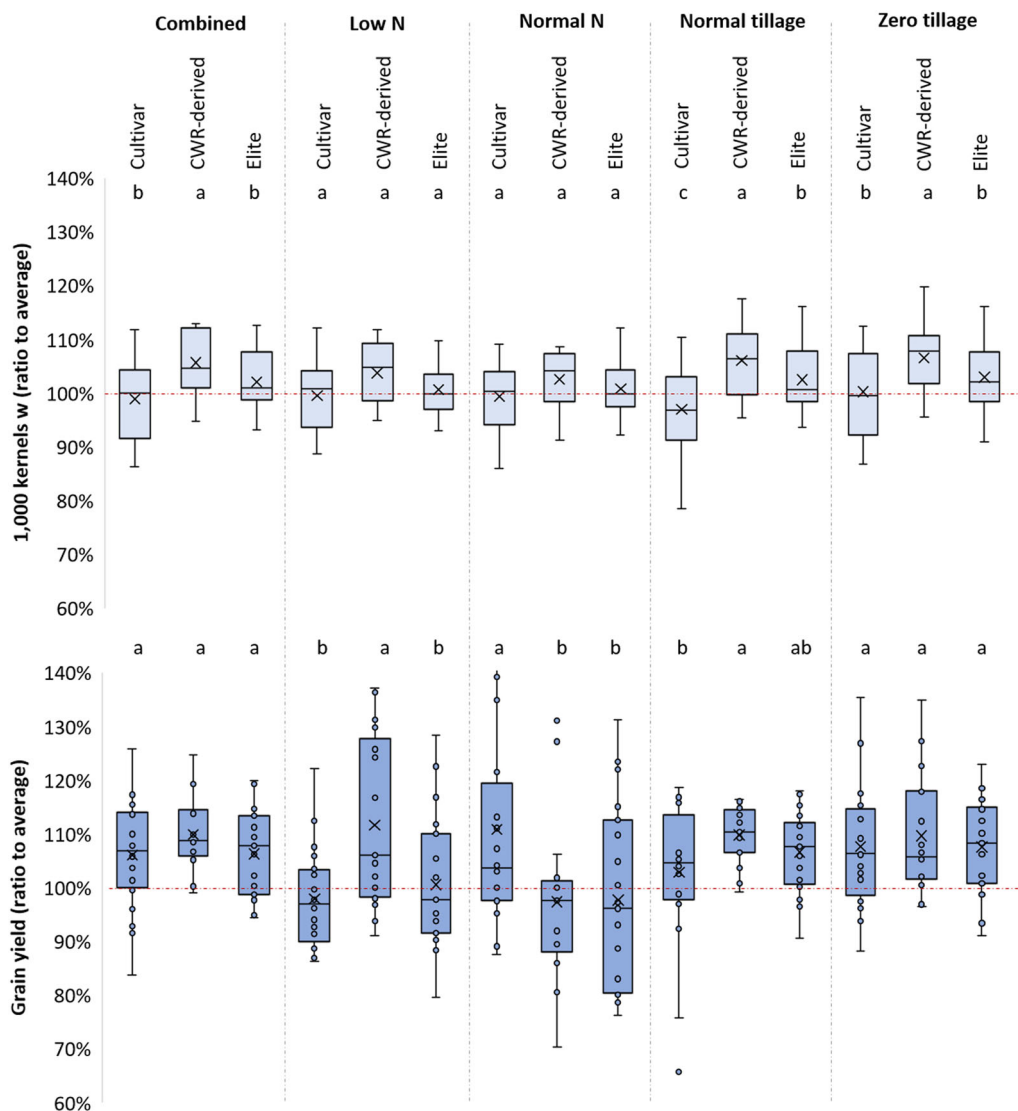


FIGURE 2 Drought tolerance screening of three germplasm types, expressed as ratio of the average performances across two seasons for 1000-kernel weight and grain yield displayed using box-and-whiskers plots. The dashed red line identifies the average of the trial (100%). The letters above the boxes were defined based on LSD

(Icavire derived from *T. araraticum*), exceeding average performances by 36–42%. For yield HTI under plastic tunnels, the best entries were Cvr-18 (Australian cultivar Saintly), Elite-07 (from CIMMYT), Elite-11 (IDON37-033), and Elite-18 (Mkilo), with values 37–45% higher than average. The top three entries with positive yield combinations for both tests were Cvr-03 (Berghouata1), Wide-13 (Icambel), and Elite-11 (IDON37-033) (see Supplemental Table S1 for details). Overall, 46% of the tested germplasm showed good levels of tolerance to heat stress based on GY. The same study conducted for the GNspk identified 42, 28, and 20% of the tested individuals as superior to the average in both experiments for CWR-derived, cultivars, and elite germplasm types,

respectively. The top GNspk lines along the Senegal River were Cvr-03 (Sudanese cultivar Berghouata1), Cvr-10 (Moroccan variety Isly), and Wide-16 (Icavire derived from *T. araraticum*), exceeding average performances by 37–45%. For GNspk HTI under plastic tunnels, the best entries were Cvr-18 (Saintly), Cvr-03 (Berghouata1), Elite-13 (IDON37-062), and Elite-04 (CaMdoH25) with values 31–66% higher than average. The top three entries with positive GNspk combinations for both tests were in order from the best: Cvr-03 (Berghouata1), Wide-16 (Icavire), and Elite-13 (IDON37-062) (see Supplemental Table S1 for details). Overall, 28% of the tested germplasm showed good levels of tolerance to heat stress based on GNspk.

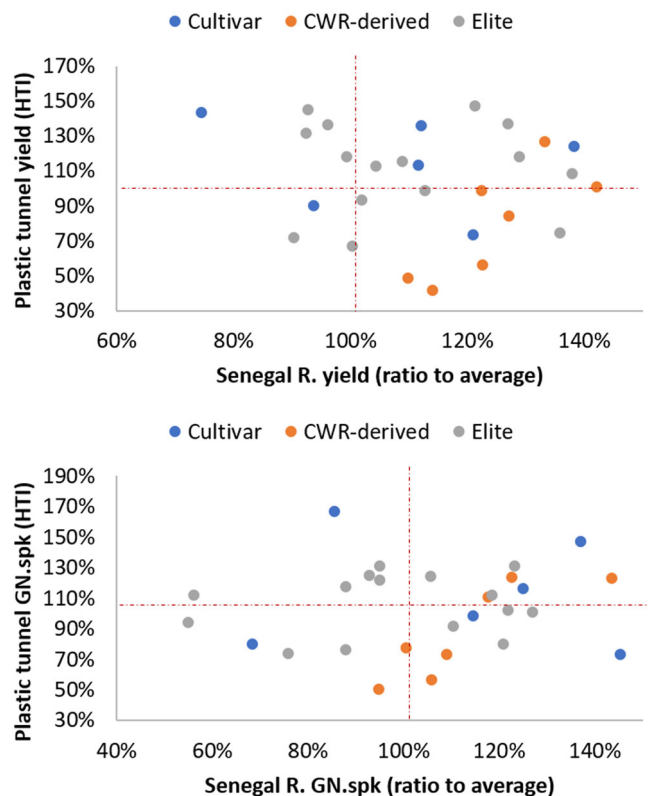


FIGURE 3 Combined test of heat tolerance by comparison to the trials means for heat tolerance index (HTI) measured as average of two seasons and two treatments (plastic tunnel for heat tolerance and normal conditions) against field results of testing at two heat-stressed sites for two seasons along the Senegal River. The dashed red lines mark the trials means

3.4 | End-use quality evaluation

End-product quality of a durum wheat cultivar is primarily determined by its SedInd obtained by combining sodium dodecyl sulfate gluten strength and protein content and YP. Seeds harvested from four environments were used to define the quality traits of the genotypes under test (Figure 4; Supplemental Table S1). The ANOVA testing revealed significant germplasm type effect ($p < .05$) for both SedInd and YP (Supplemental Table S5). The combined analysis revealed that the cultivars were significantly superior to the other two germplasm type, with averages of 101, 72, and 79% for cultivar, CWR-derived, and elite, respectively. In the case of YP, the best germplasm types were cultivar and elite at 98 and 94%, respectively, while CWR-derived were the inferior at 93% (Figure 4). The Canadian cultivar CDC Desire resulted as the best genotype for SedInd, reaching 29% above the trial mean, significantly superior ($p < .01$) to the best CWR-derived entry DWAYT-0215 (5% above trial mean) and the elite Mkilo (7% above). For YP, the best trial entry was the elite Moulisabil2 with 16%, significantly superior ($p < .01$) to the best cultivar

CDC Desire (11% above trial mean), which was also superior to the best CWR-derived entry DWAYT-0215 (1% below trial mean).

3.5 | Multitraits selection

Despite the importance of identifying germplasm sources as donors of useful traits, breeding aims, especially at the selection of the one or few best accessions, to be further promoted as cultivars. In that sense, it is of interest to define which genotype combines the most useful set of traits. The characteristics described before were then combined, genotype scores were divided into quartiles, and results for the first and second are presented in Table 3 for the top three entries of each germplasm type, and complete individuals information are reported in Supplemental Table S1. The Australian cultivar Hyperno and the Italian cultivar Svevo reached the first quartile for drought tolerance and quality characteristics and the second quartile for disease resistance, while the Senegalese cultivar Margherita was among the top accessions for heat and drought tolerance. Among the elites, IDON37-010 had top performances for drought and heat tolerance and matched the second quartile for disease resistance. Chacan instead combined disease resistance with top quality characteristics. The best entry overall was the CWR-derived Icambel, with first quartile score for drought and heat tolerance and second quartile for diseases and quality. DWAYT-0306 matched the best elite IDON37-010, while DWAYT-0215 had top quality combined with good drought tolerance and disease resistance. Depending on the specific breeding targets, different accessions should be selected but, in general, the CWR-derived Icambel appeared as the genotypes with the widest adaptation combining good response for all tested traits.

3.6 | Genetic diversity determined by minor allele frequencies

Besides the phenotypic characteristics of the different germplasm types, the use of CWRs is normally sought to widen the genetic basis of the germplasm. To test the level of genetic diversity of the three germplasm types, 22,589 polymorphic markers were assigned to four classes defined by MAF representing, in decreasing order: the frequent, common, rare, and unique alleles. The ANOVA determined a significant ($p < .01$) effect of germplasm types one MAF for the four classes of markers (Supplemental Table S6). The frequency distribution of the germplasm types was then compared with the whole panel as shown in Figure 5. For the markers with frequent minor alleles (Figure 5a),

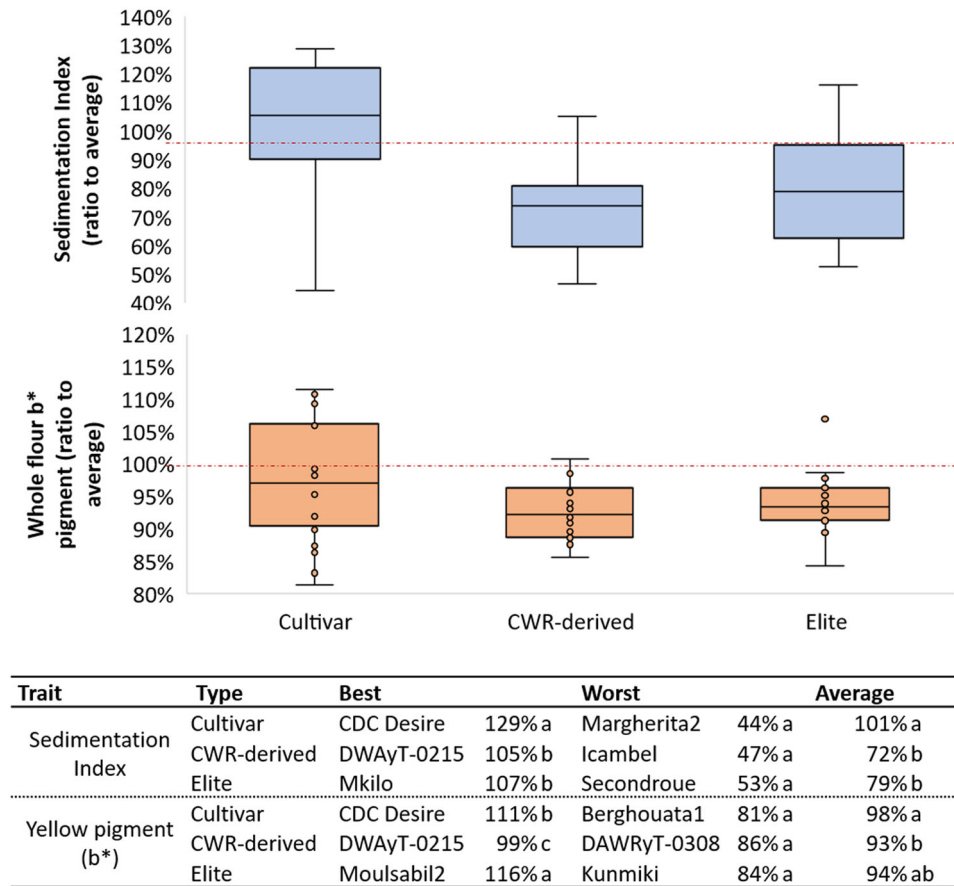


FIGURE 4 Assessment of end-use grain quality of different germplasm types. (a) Box-and-whiskers plots of the averaged across four environments for sedimentation index and yellow pigment (b*). The dashed red line identifies the average of the trial (100%). (b) The best, worst, and average performances of the germplasm types. The letters were defined based on LSD

TABLE 3 Multitraits selection of the best three accessions for each germplasm type

Name	Origin	Type	Diseases ^a	Drought ^a	Heat ^a	Quality ^a
Hyperno	Australia	Cultivar	○	●	–	●
Margherita 2	ICARDA	Cultivar	–	●	●	–
Svevo	Italy	Cultivar	○	●	–	●
Icambel	ICARDA	Crop wild relative-derived	○	●	●	○
DWAYT-0306	ICARDA	Crop wild relative-derived	○	●	●	–
DWAYT-0215	ICARDA	Crop wild relative-derived	○	○	–	●
IDON37-010	ICARDA	Elite	○	●	●	–
Chacan	ICARDA	Elite	●	–	–	●
Bezaghra	ICARDA	Elite	●	○	○	–

^a ● first quartile; ○ second quartile.

the cultivar showed a reduction of diversity compared with the whole panel with 50% of the individuals having MAF of 36–40% and no accessions falling in the top two classes. Instead, the CWR-derived had a significant increase in diversity, with 75% of the tested genotypes showing 40–44% MAF. In the case of markers with common minor alle-

les (Figure 5b), the CWR-derived lines showed a reduction in genetic diversity with 35% of the individuals reaching 15–18% MAF and none in the 31–36% class as for elites. In the case of markers tagging rare alleles (Figure 5c), skewing toward lower genetic diversity could be observed for both CWR-derived and elites. For CWR-derived, 60% of the

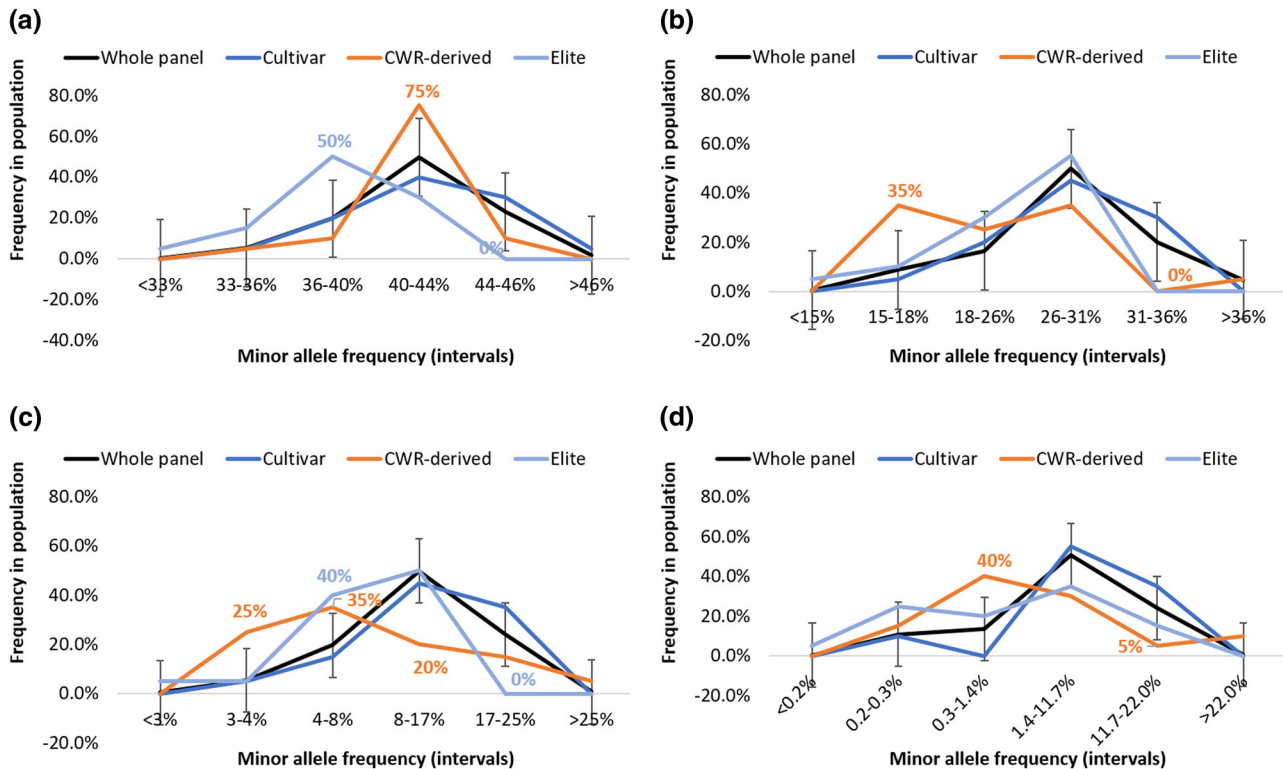


FIGURE 5 Distribution of individuals of three germplasm types compared with the whole panel (Kabbaj et al., 2017) based on minor allele frequencies (MAF) for four markers classes: (a) frequent minor alleles (30–49% MAF), (b) common (20–30% MAF), (c) rare (5–20% MAF), and (d) unique (2–5% MAF). The error bars represent the LSD value plotted on the curve corresponding to the whole durum panel described in Kabbaj et al., 2017. The intervals displayed were selected as increments of one-sixth of markers distributions for individuals

accessions felt in the first two classes, while a reduction to 20% of the individuals was recorded for the 8–17% MAF class. The individuals composing the elite set concentrated (40%) in the third class of 4–8% MAF, with none matching the two top classes of 17–25 and >25% MAF. Finally, for unique alleles (Figure 5d), the CWR-derived set also recorded a decrease of diversity compared with the whole panel with 40% of the individuals mapping in the 0.3–1.4% MAF class and only 5% in the 11.7–22% group. Overall, elites showed a significant ($p < .01$) decrease of genetic diversity vs. the whole panel for markers tagging frequent and rare alleles. No deviation from the whole-panel frequencies were observed for cultivars, while CWR-derived had an increase of genetic diversity for markers tagging frequent alleles but a reduction for all other classes.

3.7 | Genomic fraction contributed by crop wild relatives

Ten genotypes tested here were generated by first crossing an elite parental line (P_1) to a CWR and then top-crossing the resulting F_1 to a second elite parental line (P_2). In

absence of bias selection, this crossing scheme should lead to a progeny that is comprised of 25% of the genome of P_1 , 25% of CWR, and 50% from P_2 . The parental contribution was then computed as shown in Figure 6a to reveal that 56 to 65% of the loci tested were monomorphic between the two parents, and their origin could not be defined; P_1 contributed between 7 and 15% of the alleles of the progeny and P_2 15 to 19% of the alleles. Therefore, 9 to 13% of the genome of the progenies originated from the CWR, matching well ($\pm 2\%$) the fraction contributed by P_1 .

To determine the type of alleles that the CWR contributed to the resulting progeny, a MAF study based on the four classes was conducted between parents and progenies (Figure 6b). For the frequent alleles, the MAF of the progeny was inferior, matching, or slightly superior to that best parent (0.1–0.8%). For common alleles, in all cases, the CWR-derived progeny had lower MAF than the parents, while for rare and unique alleles only for one progeny an increase of 2.1 and 1.9% MAF was recorded, while all other progenies had lower value than the parents. Considering these results together and based on the genotyping analysis conducted, the addition of the CWR-derived loci did not increase the MAF and consequently the genetic

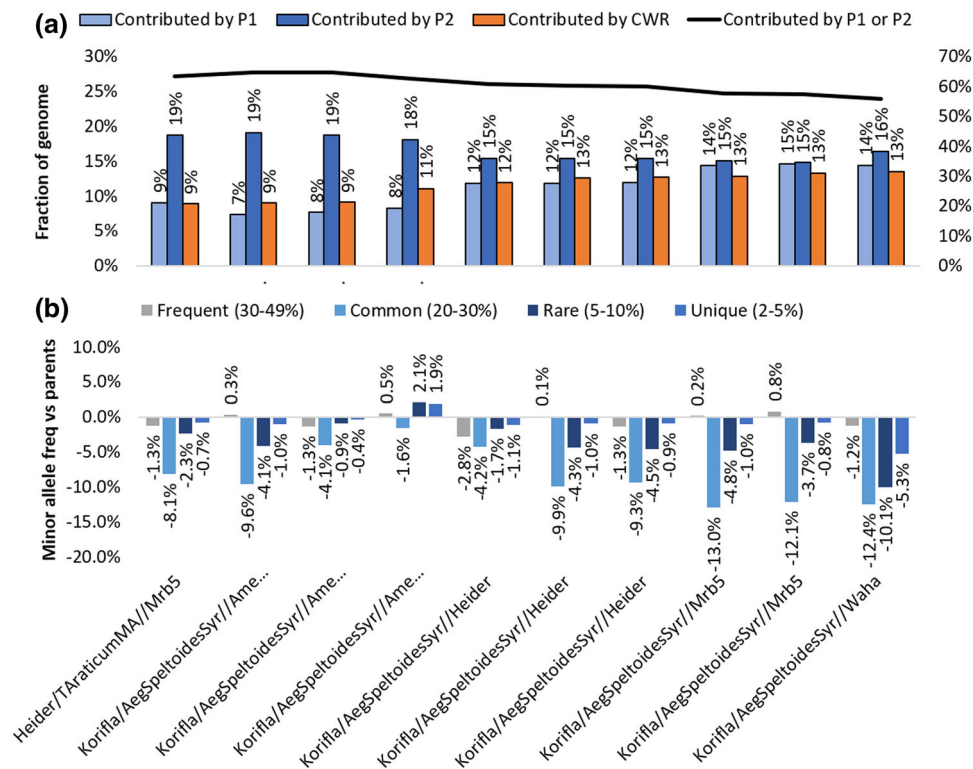


FIGURE 6 Genetic comparison of parents and resulting CWR-derived progenies for 10 top-cross lines after genotyping with 35K Axiom array. (a) Contribution of alleles in the progeny from the two parents and the CWR. For clarity of display, the alleles contributed by both parents (monomorphic) are reported on the secondary vertical axis. (b) Minor allele frequencies for four marker classes presented as comparison between the progeny and the best parent

diversity of the progenies compared with the alleles contributed by the elite parents.

4 | DISCUSSION

4.1 | Crop wild relatives as source of moderate resistance against septoria blotch

The literature reports a wide array of useful alleles that can be introgressed from CWRs to improve breeding lines (Brozynska, Furtado, & Henry, 2016; Dempewolf et al., 2017; Hajjar et al., 2007). Here, a broad phenotypic test was conducted to seek traits of interest for disease resistance, drought tolerance, heat tolerance, and end-use quality. Two germplasm types (elites and cultivars) were put in competition against CWR-derived lines to define the true superiority of using wide crosses. Two highly virulent isolates of yellow and leaf rust and aggressive strains of septoria blotch and tan spot were used to screen the germplasm over several seasons. The CWR-derived lines tested here proved to be a good source of moderate resistance against septoria blotch while showing higher susceptibility to tan spot. Conversely, for leaf rust and tan spot

the most valuable germplasm source were the elites. Interestingly, the CWR-derived set did not exhibit any MS type of response for any of the diseases screened. This probably is due to the sole presence of major genes in the CWR tested here offering only nonhost type of resistant, rather than MS response type indicative of varying levels of host-parasite compatibility. This finding is in partial disagreement with Chu, Xu, Faris, Nevo, and Friesen (2008), who identified quantitative and qualitative response to septoria blotch and tan spot when challenging accessions derived from wild emmer. Similarly, Tadesse et al. (2007) found from the evaluation of 98 CWR-derived bread wheat lines the presence of minor and major genes against tan spot. In addition, both partial and complete resistance to stem and leaf rusts were found in five D genome wild relatives of wheat (Vikas et al., 2014). Hence, the finding that no MS response type could be observed in our experiment might only be due to the specific germplasm used rather than a general trend of CWR-derived crosses.

When rusts and blotches were analyzed together, the elite germplasm displayed high frequencies of resistances to rusts, while all the three germplasm types were the same for blotches and the four diseases combined. Overall, the results for the germplasm set used here do not

allow to fully justify the use of CWR-derived breeding strategy as a major source of resistance, except in the specific case of septoria blotch. The phenomenon of a reduction in resistance to leaf rust during the transfer of trait from wild relatives was identified in several reports (Gill et al., 1986; Kema & Lang, 1992; Trotter, Jahier, & Tanguy, 1982). Recently, Zaïm et al. (2017) showed that half of her studied CWR-derived lines were immune to leaf rust contrary to tan spot for which CWRs were less resistant than the other germplasm types. Conversely, in the case of CWR-derived top crosses, the parental lines used were old elites susceptible to all four diseases, while the resulting progenies fared better. Hence, there is actual gain of resistance that can be obtained from CWR, but this was inferior to other germplasm types among the set evaluated here. From a breeding standpoint, it is probably advisable to develop CWR-derived lines that integrate the best possible elites as parents especially for resistance to tan spot. This statement seems obvious when discussing normal breeding but it is often not the case for CWR-derived lines that are commonly developed starting from common lines or old cultivars as was the case for most of the CWR-derived lines tested here.

4.2 | Crop wild relatives as an excellent source to tolerate abiotic stresses

Testing against drought stress at eight environments showed a prominent role of CWR-derived germplasm type to significantly increase the size of the grains (TKW) over cultivars and elites. This is a very important trait for durum wheat breeders as it provides a mean of adaptation against moisture stresses (Mohammadi et al., 2011; Samaan, 2007) but it is also a preferred characteristic by the food industry as larger grains have been associated with higher semolina extraction rates (Pinheiro, Costa, Almeida, Coutinho, & o Gomes, 2013; Russo et al., 2014). Several other reports have confirmed the advantage of using wild relatives to increase the grain size in durum wheat (Fritz, Cox, Gill, & Sears, 1995; Reynolds et al., 2007).

For GY under drought stress, no significant differences could be observed among germplasm types in the combined analysis, which suggests that yield performances of lines derived from CWR are as good as those of commercial cultivars and elites. The main yield advantage of CWR-derived lines could be observed at low nitrogen levels, possibly linked to the fact that CWRs typically grow in marginal lands in the absence of any forms of fertilization. This aspect is of great interest and should be studied further to see if truly CWR-derived lines are more efficient in using nitrogen than other germplasm types. Gorny and Garczynski (2008) identified a broad genotypic

variation in the efficiency of nitrogen use and response to limited fertilization in wild relatives of wheat, which are dependent upon ploidy levels. In fact, the polyploid *Triticum* species were the most efficient in nitrogen utilization; however, the diploid *Aegilops* and *Triticum* accessions were unable to use nitrogen efficiently and only *A. tauschii* (Coss.) Schmal. accessions exhibited a moderate tolerance to reduced fertilization. The same authors also suggested that wild and primitive tetraploid combined high-uptake efficiency with enhanced tolerance to nutrients shortage. Further, some wild plants can limit denitrification by releasing inhibitory secondary metabolites and some research is ongoing to transfer this characteristic to wheat (Foulkes et al., 2009; Ortiz et al., 2008; Subbarao et al., 2007).

A harsh test of heat tolerance was deployed by merging data from the heat-affected field trials carried out along the Senegal River (Sall et al., 2018a,b) and plastic tunnels used to impose a 14 °C increase in temperatures at the time of flowering. In this case, the CWR-derived lines proved equal to the other germplasm types when considering GY but superior to maintain high GNspk under heat stress. The ability to maintain fertility, and hence grains formation (GNspk), under high temperatures has been proposed as the main mechanism of durum wheat to tolerate heat stress (El Hassouni et al., 2019). It can be concluded then that CWR-derived germplasm holds advantages over the two other germplasm types for heat tolerance. Khanna-chopra and Viswanathan (1999) screened several wild relatives under heat stress and identified that wild emmer, *T. monococcum*, and *A. speltoides* Tausch var. *ligustica* (Savign.) Fiori were highly heat tolerant. Peng, Sun, Peng, and Nevo (2013) reported also that wild emmer is an important genetic resource for increasing heat and drought tolerance in wheats.

4.3 | Crop wild relative-derived germplasm not ideal for food industry

The final test was to determine the end-use quality based on four field trials and two main measurements related to gluten fraction of the grain proteins and flour color (YP). In this case, the CWR-derived lines performed worse than the other two germplasm types, with cultivars from Canada showing the best performances. This is in good agreement with the finding of Zaïm et al. (2017) that suggested to put particular care on ensuring good end-use quality when deploying wide crosses. Most authors have reported that the use of wild relatives in wheat breeding often results in poor end-use quality (Farooq & Siddique, 2017; Mondal et al., 2016; Wulff & Moscou, 2014). Our results are no exception to this trend, further stressing the importance of

using elites with excellent end-use quality as crossing parents when conducting wide crosses.

4.4 | Value of crop wild relatives for durum wheat breeding

Defining exploitable germplasm sources for improving various traits is a primary goal of prebreeding efforts, but breeders tend to seek the one or few lines that combine different traits and are ready for promotion as cultivars. A combined test of all four major traits considered here (disease resistance, drought and heat tolerance, and end-use quality) identified a CWR-derived line as the most suitable overall, reaching first or second quartile performances for all traits. While it is rare for breeders to simultaneously target all the main crop issues within one cultivar (i.e., four diseases, drought, heat, and quality), it is of academic interest to notice how it was possible to select one such cultivar only from the CWR-derived set.

Overall, the results presented here support the use CWR-derived lines as an ideal source to improve resistance to septoria blotch, drought tolerance via an increase in grain size (TKW), and heat tolerance by ensuring high spike fertility (GNspk). Moreover, it also supports the direct use of CWR-derived lines as a breeding, rather than prebreeding, method for the direct release of superior durum wheat cultivars to farmers. It needs to be noted that the CWR-derived line that outperformed all others (Icambel) was derived from a complex cross involving three hybridization with elites and *T. araraticum* (P1//P2/CWR/3/P3). Therefore, it is advisable to those breeders willing to embrace the idea of seeking new cultivars directly from wide crosses to integrate the best possible elites in the hybridization with CWR to maximize the chances of achieving superior lines.

4.5 | Improving genetic diversity via wild crosses

The 35K Axiom breeders' array was used to study the genetic diversity of the three germplasm types and compare it with what was obtained considering a much larger panel of 92 landraces and 292 modern lines from around the world (Kabbaj et al., 2017). The frequency of minor alleles is an excellent proxy to determine the abundance of genetic diversity of a set of germplasm. Typically, the most interesting alleles are those that are extremely rare (i.e. minor) and can only be found in unique germplasm sources. To mimic this, the markers of the array were divided into four classes based on their average MAF, going from those tagging the most frequent minor alleles to the most unique ones. An ideal entry to be used for increasing

genetic diversity via hybridization would be the one that has MAF for unique alleles superior to the average. Testing of this revealed that CWRs are not good sources of unique or rare alleles, with most individuals falling into low MAF classes. Instead, CWR-derived lines are particularly rich in minor alleles of the most frequent class, those that can be more easily identified in any germplasm. In fact, 75% of the individuals had MAF of 40–44% for frequent alleles, meaning that nearly all CWR-derived lines carried minor alleles in nearly half of their genome. In that sense, the CWR-derived lines are highly genetically diverse. A direct comparison between parents and progenies of CWR-derived lines confirmed that 8–13% of the CWR genome is retained in the progenies under breeding selection pressure, a value that match what recorded for P₁ alleles. However, the CWR genome fraction did not consistently increase the overall genetic diversity (MAF) as compared with the parents.

A significant fraction of the CWR genome is therefore retained in the final progenies and contributes to improve several phenotypic characteristics but it does not appear to increase the genetic diversity based on MAF analysis except for frequent alleles. The most obvious explanation can be found in the specific type of genotyping platform used. The Axiom 35K breeders' array was specifically designed to maximize the detection of polymorphism between elite lines and cultivars, so it might not be suitable to identify CWR alleles. Still, it is worth mentioning that in the study by Kabbaj et al. (2017), this same array allowed discriminating the strong genetic diversity harbored by landraces over modern germplasm. Thus, the genotyping platform used might not be the only explanation, as it alone fails to justify why MAF of frequent alleles increased in CWR-derived lines. Could it be that changes in methylation occur when CWR are hybridized to modern durum parents, and the phenotypic effects observed are the result of an epigenetic effect rather than a direct introgression of wild alleles? A similar hypothesis was proposed in interspecific hybrids of *Solanum tuberosum* L. × *Solanum kurtzianum* Bitter & Wittm., where methylation changes were identified with higher frequency than allelic changes (Marfil, Masuelli, Davison, & Comai, 2006). Other authors reported the possibility of similar epigenetic effects when working with alien introgressions in wheat, with some entries that achieved better phenotypic response vs. their donor elite parents even after the loss of the alien introgressions as a result of recombination (Kuzmanovic, Rossini, Ruggeri, Pagnotta, & Ceoloni, 2020, Zhang et al., 2008). As daunting and tempting as this question is, specific research will be required to truly grasp the extent of DNA reconfiguration that might occur in CWR-derived lines. Still, it remains an interesting hypothetical tool that durum breeders have yet to deploy in an effective manner and could

further add to the power of using CWR in their crossing schemes.

The results of this study showed the value of incorporating wild relatives in durum wheat breeding for enhancing genetic diversity of frequent alleles, improving resistance to some foliar diseases, and improving tolerances to major abiotic stresses. No evident linkage drag was observed in CWR-derived lines in terms of agronomic performances but some care should be taken when selecting the elite parental lines for the hybridization by prioritizing good end-use quality.

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AUTHOR CONTRIBUTIONS

EHN, KH, and FMB wrote the article; FMB, RO, MB, and AA designed the research; AA and FMB produced the CWR-derived lines; KH, ZM, EHK, SAT, AM, and GF conducted the experiments; all authors reviewed and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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SUPPORTING INFORMATION

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