



Recent Progress in Germplasm Evaluation and Gene Mapping to Enable Breeding of Drought-Tolerant Wheat

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There is a need to increase wheat productivity to meet the food demands of the ever-growing human population. However, accelerated development of high yielding varieties is hindered by drought, which is worsening due to climate change. In this context, germplasm diversity is central to the development of drought-tolerant wheat. Extensive collections of these genetic resources are conserved in national and international genebanks. In addition to phenotypic assessments, the use of advanced molecular techniques (e.g., genotype by sequencing) to identify quantitative trait loci (QTLs) for drought tolerance related traits is useful for genome- and marker-assisted selection based approaches. Therefore, to assist wheat breeders at a critical time, we searched the recent peer-reviewed literature (2011-current), first, to identify wheat germplasm observed to be useful genetic sources for drought tolerance, and second, to report QTLs associated with drought tolerance. Though many breeders limit the parents used in breeding programs to a familiar core collection, the results of this review show that larger germplasm collections have been sources of useful genes for drought tolerance in wheat. The review also demonstrates that QTLs for drought tolerance in wheat are associated with diverse physio-morphological traits, at different growth stages. Here, we also briefly discuss the potential of genome engineering/editing to improve drought tolerance in wheat. The use of CRISPR-Cas9 and other gene-editing technologies can be used to fine-tune the expression of genes controlling drought adaptive traits, while high throughput phenotyping (HTP) techniques can potentially accelerate the selection process. These efforts are empowered by wheat researcher consortia.

Keywords: drought tolerance, genetic resources, landraces, quantitative trait loci mapping, wheat, climate change

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the world's major cereal crops with global production of 756.7 million tons in 2017 (FAO, 2018). The world's population is expected to exceed 9 billion by 2050, requiring at least a 60% increase in wheat yield (United Nations, 2019). An increase in wheat yield from the current level of 1% per year to at least 1.6% is deemed necessary to address this challenge (GCARD, 2012). This is further challenged by projected unpredictable rainfall patterns

associated with climate change which are expected to lead to more drought events (IPCC, 2013). However, the degree of impact on final yield depends on the growth stage and the intensity and duration of stress events (Daryanto et al., 2016; Sarto et al., 2017).

Like many other crops, drought affects wheat at all growth stages (HongBo et al., 2005; Saeidi et al., 2015; Saeidi and Abdoli, 2015; Wang et al., 2015; Sarto et al., 2017; Ding et al., 2018). Some of the growth stage-specific physio-morphological traits associated with drought tolerance in wheat include: early vigor (Rebetzke et al., 1999), coleoptile length (Rebetzke et al., 2007), leaf chlorophyll content (Khayatnezhad et al., 2011; Kira et al., 2015; Ramya et al., 2016), glaucousness (waxiness) for photo-protection (Merah et al., 2000; Bi et al., 2017), leaf rolling (Kadioglu and Terzi, 2007), carbon isotope discrimination (Kumar and Singh, 2009), flag leaf senescence (Verma et al., 2004; Hafsi et al., 2013) and plant height (Su et al., 2019). Also, root system architecture (RSA) traits are fundamental targets for breeding drought-tolerant wheat varieties (Lopes and Reynolds, 2010). A breeding program that selects for these physio-morphological traits has the potential to contribute to drought stress tolerance in wheat, as recently reviewed (Khadka et al., 2020).

In general, breeding can be accelerated by exploiting the diversity of genetic resources as sources of alleles that enhance desirable traits. More than 800,000 wheat accessions, including local landraces and synthetics, are conserved in genebanks globally (FAO, 1998). A large proportion of these accessions are accessible to wheat breeders. Recent advancements in genomics (e.g., genotype by sequencing, see below) have enabled exploration of this genetic diversity, leading to the discovery of markers and associated quantitative trait loci (QTLs) that can be utilized in marker assisted selection and genomic selection to accelerate variety development (Huang and Han, 2014). Wheat has a large and complex genome (Marcussen et al., 2014; Shi and Ling, 2017; Uauy, 2017; Abrouk et al., 2018). Very recently, however, an annotated reference whole genome sequence for bread wheat was released, which described 107,891 high-confidence level genes (IWGSC, 2018). This release has provided a significant opportunity to use genetic resources for exploring the wheat genome, and selecting alleles that encode desirable physio-morphological traits associated with drought tolerance.

There have been excellent recent reviews on the progress in breeding wheat and other cereals for tolerance to abiotic stress including drought (Mohammadi, 2018; Choudhary et al., 2019; Gupta et al., 2020). However, a unique feature of this current paper is that it reviews the different genetic resources that have been or can be exploited to accelerate the breeding of wheat for drought tolerance. The paper comprehensively updates recent progress (2011–2020) on the discovery of QTLs that promote drought tolerance in wheat, expands the list of associated physio-morphological traits and provides helpful details to breeders (e.g., QTL source populations). The QTL section is now the most up-to-date review on the topic. Furthermore, this paper also presents very recent advances in genetic engineering including

the CRISPR/Cas9 genome editing system that has been used to target genes conferring drought tolerance.

UTILIZATION OF GENETIC VARIATION FROM DIVERSE SOURCES

Use of diverse germplasm is key to the development of drought-tolerant wheat varieties. As noted in **Table 1**, *T. aestivum* landraces are one of the major groups of genetic resources valuable for breeding drought-tolerant wheat (Mwadzingeni et al., 2017). They have complex morphological diversity and are mostly grown in low input environments (Padulosi et al., 2012) which make them more adapted to stress (Padulosi et al., 2012; Lopes et al., 2015a). For example, a group of Creole wheat landraces (the landraces introduced to Mexico from Europe) showed better adaptation to different abiotic stresses, including drought, due to the presence of rare but beneficial alleles (Vikram et al., 2016). Similarly, the Japanese landrace “Aka Komugi” is one of the sources of the dwarfing *Rht8c* allele (Lopes et al., 2015a; Grover et al., 2018) that contributes to breeding drought tolerant wheat. This is because *Rht8c* promotes a higher-yielding semi-dwarf phenotype, but does not reduce the length of the coleoptile and thus permits deep sowing (Lopes et al., 2015a). Furthermore, in a study that evaluated 21 genotypes, nine wheat landraces exhibited drought tolerance based on a stress susceptibility index (SSI) (Sareen et al., 2014).

In addition to landraces of *T. aestivum*, other domesticated wheat species such as *T. compactum* Host, *T. sphaerococcum* Perc., *T. durum* Desf, *T. turgidum* T. *turanicum* Jakubz (“Kumut”) and *T. polonicum* L., are also sources of valuable alleles to enable breeding of wheat for drought tolerance (Janni et al., 2018; Szabo-Hever et al., 2018; Guellim et al., 2019; Nemtsev et al., 2019). Wild relatives of wheat and other related genera such as *Aegilops* are additional valued sources of germplasm for breeding drought-tolerant varieties (**Table 1**).

The rye (*Secale cereale* L.) derived wheat genotypes, also known as the 1B/1R chromosome translocation lines, have been utilized by different wheat breeding programs (Kumar et al., 2003; Hoffmann, 2008; Ren et al., 2017). These 1B/1R chromosome translocation genotypes consist of the long arm of wheat chromosome 1B including its centromere, and the short arm or a portion of rye chromosome 1R (Cai and Liu, 1989; Heslop-Harrison et al., 1990). These genotypes are also important sources of abiotic stress tolerance including drought-tolerance (Kumar et al., 2003; Rajaram, 2005). Hoffmann (2008) demonstrated that the 1B/1R translocation wheat genotypes possess greater drought tolerance. However, this may further depend on the genetic background of the recipient wheat genotype (Monneveux et al., 2003; Singh et al., 1998) as shown by Tahmasebi et al. (2015) where 1B/1R translocation genotypes were not suitable sources of diversity for improving drought tolerance.

The challenges in utilizing these landraces and wild relatives are often associated with epistatic and pleiotropic effects of some genes, leading to linkage drag (Sehgal et al., 2015). However,

TABLE 1 | Recent peer reviewed reports (2011-current) of germplasm evaluation for assessing drought tolerance in wheat.

#	Collaborating institutions	Experiment/evaluation	Germplasm type	Number of genotypes	Drought treatment	Results	Reference
1	Bread Wheat Breeding Program, CIMMYT	Evaluation of high yielding genotypes using data from 740 international Semi-Arid Wheat Yield Trials (SAWYT) conducted in 66 countries from 2002–2003 to 2013–2014 was performed to determine genetic gain for grain yield.	The SAWYT included advanced breeding lines including SHW derivatives and local checks.	50 entries in each SAWYT	Experiments conducted using local management practices. After 2 year testing under optimum irrigation and 1–2 years of testing under drought and heat stress, the elite lines were selected.	Results showed broader use of genotypes Pastor, Baviacora 92, and synthetic hexaploid derivatives to develop stable and drought-tolerant wheat lines.	(Crespo-Herrera et al., 2018)
2	University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa Agricultural Research Council-Small Grain Institute, Bethlehem, South Africa University of South Africa, Pretoria, South Africa	A panel of wheat genotypes were tested under greenhouse and field conditions during 2014/15 and 2015/16.	Advanced CIMMYT lines and local checks	96 lines were tested (including 88 lines from CIMMYT's heat and drought nurseries and 8 local checks).	Drought stress treatments were applied after heading until maturity by withholding irrigation to 35% of field capacity	Twelve CIMMYT lines were selected as drought-tolerant after the evaluation.	(Mwadingeni et al., 2016b)
3	Razi University, Kermanshah, Iran	A set of wheat genotypes were evaluated under rain-fed and normal irrigated conditions in 2010–11 season using 13 drought tolerance indices.	Landraces	12	The drought stress was applied by supplying no irrigation, while the non-stressed treatments were provided with irrigation.	The landraces WC-4953S, WC-47572, and WC-47574 were identified as drought-tolerant.	(Farshadfar et al., 2012)
4	Kerman University, Iran	The wheat genotypes previously reported as drought-tolerant, were evaluated for drought tolerance using nine different drought indices under normal and water stressed conditions for two seasons in 2009–10 and 2010–11.	Landraces and modern cultivars	40	Cyclic drought stress was applied in a glasshouse experiment. The field experiments were conducted under fully irrigated and rainfed conditions.	Landrace Mahdavi was identified as the most drought-tolerant genotype.	(Abdolshahi et al., 2013)
5	Indian Institute of Wheat and Barley Research, Karnal, India	The wheat genotypes representing major wheat growing zones in India were tested for two seasons under different water regimes using the three marked water stress indices.	Modern cultivars	15	Normal (5 irrigations) and restricted irrigation treatments were applied to assess drought tolerance.	Three genotypes, NI-5439, WH-1021, and HD-2733, were identified as the most drought-tolerant cultivars.	(Meena et al., 2015)
6	University of Reading, United Kingdom	A set of wheat genotypes were assessed for drought tolerance in an ambient glasshouse environment using different drought indices.	Modern cultivars and a local check	6	Three watering regimes (100%, 35%, and 25% capacity) were used to test for drought tolerance.	Hashim-8 was identified as the superior variety for drought tolerance.	(Khakwani et al., 2011)
7	Mansoura University, Mansoura, Egypt	The wheat genotypes were evaluated for the effect of two levels of osmotic stress at seedling stage.	Modern cultivars (released varieties from 1999 to 2011)	10	The treatments were normal irrigation until 45 days, and irrigation withheld for 21 days in the stress treatment.	The results showed a negative effect of the stress on morphological seedling traits. However, variation was observed among the genotypes. Sids 13, one of the tested genotypes, was the most drought-tolerant, while Shandawel 1 was observed to be the most sensitive genotype.	(Mickky and Aldesuquy, 2017)

(Continued)

TABLE 1 | Continued

#	Collaborating institutions	Experiment/evaluation	Germplasm type	Number of genotypes	Drought treatment	Results	Reference
8	Mansoura University, Mansoura, Egypt University of Debrecen, Hungary	Hungarian wheat landraces were selected and tested for different physiological traits at the seedling stage.	Landraces	7	Five levels of water stress were applied on seedlings (0%, 6%, 12%, 18%, and 24%) using PEG-6000.	Two landraces, Leweucei and Mateteleki, were found to be more drought-tolerant based on different drought tolerance related parameters such as higher relative water content (RWC), tolerance index (TI) and activities associated with α and β -amylases.	(Abido and Zsombik, 2018)
9	Nanjing Agricultural University, PR China	One drought-tolerant (Luhan-7) and one drought sensitive (Yangmai-16) variety were evaluated to assess improved tolerance to water stress during post-anthesis growth phase as a result of pre-drought priming at different stages during the vegetative growth phase.	Modern winter wheat cultivars	2	Drought priming was done at tillering and jointing stages with moderate stress (55–60% of field capacity). Severe stress was applied at 35–40% field capacity, seven days after anthesis.	Results showed positive effect of drought priming on both varieties when they were exposed to post-anthesis drought. However, the drought-tolerant genotype showed greater response to priming, while the growth stage of priming also contributed to drought tolerance to some extent.	(Abid et al., 2016)
10	Cereal Research Non-Profit Ltd., Hungary Snowy River Seeds Pty Ltd., Australia	Wheat genotypes were phenotyped for different root and shoot traits under drought in a glasshouse along with one drought-tolerant and one drought susceptible check.	Modern cultivars (released varieties)	29	The well-watered pots were irrigated to 60% soil water capacity, while the drought stress pots were irrigated to 20% soil water capacity.	Based on final grain yield, three varieties were identified as drought-tolerant.	(Nagy et al., 2018)
11	Punjab Agricultural University, Ludhiana, India	A group of genotypes consisting of 57 <i>Aegilops tauschii</i> accessions and 26 <i>Triticum dicoccoides</i> accessions were used to assess adaptive plasticity induced by water stress for different morpho-physiological characters such as root-shoot development, induction of proline and cell membrane injury.	Wild relatives	83	PEG based water stress was imposed at different concentrations (10, 15, 20, and 25%).	Some of the <i>Ae. tauschii</i> accessions such as 9816, 1409, and 14128, and <i>T. dicoccoides</i> accessions 5259 and 7130, exhibited significantly higher adaptive plasticity for water stress.	(Suneja et al., 2019)
12	The University of Western Australia, Perth, Australia The Bangladesh Agricultural Research Institute, Gazipur, Bangladesh	Near isogenic lines (NILs) derived from the cross between C306 and Dharwar Dry, two varieties with spring growth habit, were evaluated targeting a QTL on chromosome 4BS that confers drought tolerance.	Putative NILs pairs	10	A glasshouse experiment was performed by maintaining 80% field capacity of moisture in the control treatment, and no water was supplied for 7 days after anthesis in the stress treatment.	Results showed that NILs having C306 background out-performed the NILs with the Dharwar Dry background, while one isoline qDS1.4B.1-10(-) that carried an allele from Dharwar Dry showed superiority over the corresponding isoline with the C306 background.	(Mia et al., 2019)
13	Northwest Agricultural and Forestry University, Yangling, China	Wheat genotypes representing important wheat growing areas in China was evaluated in irrigated and water-limited environments using rainout shelters for 14 traits, including morpho-physiological and yield traits to distinguish genotypes tolerant to drought stress.	A representative collection from 328 winter wheat accessions	90	The experiment was conducted under rainout shelters. The control plots were provided with a total 2000 m ³ /ha of water at different growth stages while the drought stress plots were provided with only 1200 m ³ /ha.	Five genotypes were identified as highly drought.	(Chen et al., 2012)

(Continued)

TABLE 1 | Continued

#	Collaborating institutions	Experiment/evaluation	Germplasm type	Number of genotypes	Drought treatment	Results	Reference
14	Northwest Agricultural and Forestry University, Yangling, China	The wheat alien chromosome addition lines derived using Chinese Spring as the common parent, were evaluated using 10 important agronomic traits under irrigated and water-limited conditions.	Landrace derived during the advanced lines	82	The control and drought stress treatments were provided with a total of 210 mm and 120 mm irrigation, respectively, in a two year evaluation using rainout shelters.	The result showed that 26 out of 82 lines possessed high levels of drought tolerance.	(Liu et al., 2015)
15	China Agricultural University, Beijing, China Ministry of Agriculture, Xinjiang, China Xinjiang Academy of Agricultural Sciences, Xinjiang, China Chinese Academy of Sciences, Beijing, China	Two season (2011 and 2012) evaluation was performed involving wheat genotypes from the CIMMYT Wheat Physiological Germplasm Screening Nursery (CWPGSN) and seven Chinese local spring wheat genotypes. The goal was to identify the most stable genotypes across water-stressed and controlled conditions.	Local genotypes and CIMMYT advanced lines	145	Water was supplied by drip irrigation. In the no-stress condition, irrigation was supplied seven times and 8 times during 2011 and 2012, respectively. In the drought stress condition, irrigation was supplied only two and three times during 2011 and 2012, respectively.	Seven lines from CWPGSN and three local varieties (Xinchun 11, Xinchun 23 and Xinchun 29) were found to be the most stable.	(Zhang et al., 2019)
16	Bulgarian Academy of Sciences, Sofia, Bulgaria Slovak University of Agriculture, Nitra, Slovak Republic	Seedlings of six modern semi-dwarf and six old tall genotypes were evaluated for drought stress tolerance.	Modern cultivars and landraces	12	The seedlings were subjected to stress by withholding water for six days starting 14 days after planting.	The results indicated that the modern varieties were more drought-tolerant, as the water balance maintenance was better compared to the old varieties.	(Petrov et al., 2018)
17	University of Idaho, Aberdeen, USA Northwest Agricultural and Forestry University, Yangling, China Institute of Water Saving Agriculture in Arid Regions of China, Yangling, China USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, USA	Assessment of wheat genotypes for drought tolerance under irrigated and terminal drought environment was performed in the 2012–2013 and 2013–2014 seasons.	Winter wheat accessions from the USDA-ARS National Small Grains Collection	198	The terminal drought stress was imposed by stopping irrigation after heading.	Based on drought susceptibility index and membership function value of drought tolerance, 23 accessions were reported to have drought tolerance.	(Liu et al., 2017)
18	University of Faisalabad, Punjab, Pakistan University of Islamabad, Islamabad, Pakistan Yunnan Academy of Agricultural Sciences, Kunming, China	A diversity panel of bread wheat genotypes was used to assess the selection criteria for drought tolerance at the seedling stage. Seedling traits, including root length, fresh weight, dry weight cell membrane thermo-stability and chlorophyll b, were suggested to improve genetic gain for drought tolerance.	A panel of landraces, historical Pakistani varieties and advanced breeding lines	105	Greenhouse experiments were conducted by maintaining 100% and 50% of field capacity in the non-stress and drought stress treatments, respectively.	Out of these 105 genotypes, 10 drought-tolerant genotypes were identified.	(Ahmed et al., 2019)

(Continued)

TABLE 1 | Continued

#	Collaborating institutions	Experiment/evaluation	Germplasm type	Number of genotypes	Drought treatment	Results	Reference
19	Imam Khomeini International University, Qazvin, Iran Ilam University, Ilam, Iran The University of Western Australia, Perth, Australia	A panel of wild relatives of wheat were tested under irrigated and water-stressed conditions.	Wild relatives of wheat	180	A greenhouse pot experiment was conducted by applying drought stress after the three-leaf stage by subjecting treatments to 30% field capacity compared to 100% field capacity for the control.	The result showed that 12 accessions were highly superior in drought tolerance. One remarkable observation was that <i>Ae. speltoides</i> (suggested source of B genome) and <i>Ae. tauschii</i> (source of D genome) responded very well to drought stress.	(Ahmadi et al., 2018)
20	Agricultural Research, Education and Extension Organization (AREEO), Iran	A set of landraces, with the majority being of Iranian origin, were tested under irrigated and drought treatments.	Landraces	97	The field experiment was conducted by applying regular irrigation for the control, while irrigation was stopped at heading in the stress treatment.	Based on the drought indices used in the evaluation, superior genotypes for drought tolerance were identified.	(Arshad et al., 2016)

these unfavorable genetic effects can be minimized through recurrent backcrossing with elite genetic materials and selection. In general, the development of modern varieties has reduced the genetic diversity of bread wheat, and this reduction in diversity shows both spatial and temporal trends (Rauf et al., 2010). Studies have shown that there is extensive reduction of nucleotide diversity in the A and B genome in modern wheat, including elite lines, compared to their progenitors (Haudry et al., 2007; Dreisigacker et al., 2008). Nonetheless, elite germplasm is a convenient source of genetic variation and has been considerably utilized in breeding for drought-tolerant wheat (Table 1). Most of the breeding programs around the world work on elite materials to develop drought-tolerant cultivars despite the advantages that landraces and wild relatives potentially carry. The major reason behind this is that in elite materials, there is less linkage drag associated with co-inheritance of undesirable and defective genes and rare alleles. As a result, elite materials have fewer challenges related to pleiotropic and epistatic gene effects in breeding populations (Sehgal et al., 2015; Mwadzingeni et al., 2017). The advantages offered by modern elite genetic materials compared to landraces and wild relatives make enhancement of genetic gain and selection response more rapid.

As a way to move forward, synthetic hexaploid wheats (SHWs) have been developed to widen the genetic variation in modern wheat. SHWs have deployed ancestral genomes using interspecific hybridization techniques (Reynolds et al., 2007; Khan et al., 2016). The SHW lines (AABB'D') have been developed by artificial hybridization of tetraploid durum wheat (*T. turgidum*, AABB) and diploid wild goat grass (*Aegilops tauschii*, D'D') (Reynolds et al., 2007; Jafarzadeh et al., 2016; Khan et al., 2016; Li A. et al., 2018; Rosyara et al., 2019). A study involving CIMMYT SHWs has demonstrated the importance of the D' genome of *Ae. tauschii* in maintaining genetic diversity as well as improving genetic gain (Rosyara et al., 2019). The diversity in the D' genome may be contributing to increased stress tolerance in SHW lines (Khan et al., 2016). In one study, six SHW lines were compared to four winter wheat cultivars grown in the U.S. Great Plains under drought stress (Becker et al., 2016). One of the SHW lines was shown to be superior under drought conditions for root morphological traits including deep root biomass and length of the longest root. In another study, an evaluation of 33 *Ae. tauschii* accessions and corresponding SHW lines revealed a wide range of variability for drought tolerance (Sohail et al., 2011). CIMMYT's efforts to develop SHW lines based on 600 genebank *Ae. tauschii* accessions has been successful, as indicated by the encouraging adoption of SHW cultivars in India and South-West China; of these, at least 30% have been reported to be drought-tolerant (Aberkane et al., 2019). Similarly, Song et al. (2017) found that six out of 34 SHW lines were drought-tolerant, and these SHW lines showed high antioxidant activities (superoxidase and peroxidase activities) that minimize drought-associated oxidative cellular damage, and thus improve drought tolerance (Zhang and Kirkham, 1994; Laxa et al., 2019). In this study, the SHWs also demonstrated superior drought tolerance over the

parental lines (Song et al., 2017). Similarly, Lopes and Reynolds (2011) observed that under drought conditions, four synthetic hexaploid derived lines out-yielded the parental lines by an average of 26%. Combined, these studies suggest that there are opportunities to exploit broad genetic variation in SHWs to improve different agronomic traits including drought tolerance in bread wheat.

Furthermore, mutation breeding, including using elite germplasm, can be another avenue to create variants suitable for drought adaptation. Among the cereals, wheat is one of the major crops in which mutation breeding has been employed for cultivar development. For example, “Sharbati Sonora,” an early maturing wheat cultivar developed by gamma radiation of a Mexican cultivar, made a major contribution to wheat production in India (Raina et al., 2016). Using gamma ray radiation, 11 drought-tolerant wheat mutant lines were identified in a recent study (Sen et al., 2017). Although mutation breeding is less common compared to other breeding methods, it has potential to generate novel stress tolerance alleles.

The major sources of the above mentioned genetic resources are national and international genebanks that maintain extensive collections of wheat landraces, wild relatives, breeding populations, obsolete varieties, and modern elite varieties. The diversity of wheat germplasm deposited in these genebanks appears to be under-utilized in breeding wheat for drought tolerance, though there are growing initiatives to evaluate and identify accessions that are suitable for wheat breeding programs. These initiatives include: The Wheat Pre-breeding Project (<https://www.cwrdiversity.org/partnership/wheat-pre-breeding-project/>), The Wheat Improvement Strategic Programme (WISP) Consortium (<http://www.wheatisp.org/Consortium/WISP.php>) and Seeds of Discovery (SeeD) (<https://seedsofdiscovery.org/>) (Singh S. et al., 2018). Genesys (<https://www.genesys-pgr.org/welcome>) is an online portal that hosts information about plant genetic resources deposited in genebanks. Out of almost 320,000 wheat accessions documented in Genesys, 23% are landraces, and 7% are wild relatives. The International Maize and Wheat Improvement Centre (CIMMYT) (<http://www.cimmyt.org/seed-request/>) hosts the most extensive collection of wheat germplasm (102,375 accessions). USDA-ARS genebanks hold 67,615 wheat accessions: most of them are maintained at the National Small Grains Collection (<https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-grains-and-potato-germplasm-research/docs/national-small-grains-collection/>) in Aberdeen, ID. Wild species are held at the Wheat Genetic Resource Center (WGRC) (<https://www.k-state.edu/wgrc/>), and additional materials at the Germplasm Resources Information Network (GRIN) (<https://www.ars-grin.gov/>). The Australian Grains Genebank (<http://www.seedpartnership.org.au/associates/agg>) has 42,624 accessions, and the International Center for Agriculture Research in the Dry Areas (ICARDA) (<http://www.icarda.org/>) has 41,471 accessions. ICARDA and CIMMYT together are managing 1,570 accessions of *Ae. tauschii* (Aberkane et al., 2019). Other organizations maintaining wheat germplasm are: Navadanya in India (<http://www.navdanya.org/>), the Svalbard Global Seed Vault in Norway (<https://www.croptrust.org/our->

work/svalbard-global-seed-vault/) and the National Bureau of Plant Genetic Resources (NBPGR) in India (<http://www.nbpgr.ernet.in/>). However, a limitation associated with the Svalbard, Navadanya, and NBPGR collections is their restricted access.

Once genetic diversity has been identified for crossing, a double haploid (DH) strategy can be employed. The DH lines possess identical copies of chromosomes in the genome which are complete homozygotes: this allows quick fixation of desired alleles. Wheat DH populations (Fleury et al., 2010; Yan et al., 2017) show potential in improving drought tolerance, as they enhance selection efficiency by improved additive genetic variance for complex quantitative traits (Dashti et al., 2007; Mwadzingeni et al., 2017). The DH lines are considered important for QTL x environment interactions, as trait means are estimated more efficiently, resulting in precise selection due to complete homozygosity (Shamasbi et al., 2017). DH breeding, augmented by advanced molecular tools, has potential to contribute to breeding drought-tolerant wheat. Different studies in wheat have shown the potential of DH lines as sources of stress tolerance. For example, Fatima et al. (2018) identified five drought-tolerant DH lines out of 84, which were tested under control and drought stress conditions. However, DH lines are associated with negative effects from colchicine treatment, and also gametoclonal and somaclonal variation that affect plant performance. Furthermore, a high level of homozygosity may make DH lines inferior to conventionally developed inbred lines (Niemirowicz-Szczytt, 1997). Other major challenges associated with DH in breeding for drought tolerance are financial expense and unique technical expertise involving plant cell and tissue culture techniques.

MOLECULAR BREEDING OF WHEAT FOR DROUGHT TOLERANCE

Identification of QTLs for Drought Tolerance Related Traits

By employing the above genetic diversity, conventional linkage mapping, using biparental populations and double haploid lines, has commonly been used to locate QTLs/genes associated with target traits, including those associated with drought (Sallam et al., 2016; Zhao et al., 2018; Li L. et al., 2019). The objective of these studies is to facilitate marker/genome assisted selection. As the mapping populations used in traditional linkage mapping are derived by hybridization between two parents (most cases), and have limited genetic variation, only low natural allelic diversity can be captured which results in low resolution QTLs (Sallam et al., 2016; Zhao et al., 2018). Furthermore, the large genome size of wheat, which has different epistatic interactions among the QTLs, in addition to a large number of genes influencing a trait, have reduced the identification of useful QTLs (Ashraf, 2010). However, more recently, genome-wide association studies (GWAS) studies have been used to identify marker-trait associations for the trait(s) of interest (Corvin et al., 2014; Scherer and Christensen, 2016). GWAS represents a powerful alternate to linkage mapping. GWAS facilitates the exploration

of the genetic variation of complex quantitative traits such as drought, controlled by several genes and their interactions (Kooke et al., 2016). GWAS exploits linkage disequilibrium (LD) resulting from variants at a locus caused by different factors such as historical mutations, natural and artificial selection, and other forces (Wang et al., 2012; Huang and Han, 2014; Visscher et al., 2017). The method accommodates natural populations with diverse genetic backgrounds (Huang and Han, 2014) and hence takes advantage of the diversity available in the genebanks described above. The efficiency of GWAS depends upon individual factors such as the number of loci for a trait that segregates in the population, genetic architecture, and size of the study population (Visscher et al., 2017). For more precise detection of QTLs/genes, some studies have combined linkage mapping and GWAS (Sallam et al., 2016; Shi W. et al., 2017; Liu et al., 2018; Zhao et al., 2018, Li G. et al., 2019).

GWAS and linkage mapping of drought tolerance QTLs have been enabled by the availability of a high-quality reference genome for common wheat along with next-generation sequencing (NGS) technologies (Kilian and Graner, 2012; Ray and Satya, 2014; Ramirez-Gonzalez et al., 2015; IWGSC, 2018). Genotype by sequencing (GBS), a DNA sequencing method that follows the NGS protocol, does not require prior genome sequence information and has enormous potential to genotype complex genomes such as in wheat (Poland et al., 2012; Mwadzingeni et al., 2016a; Chung et al., 2017).

Using these methodologies, important QTLs for wheat physio-morphological traits associated with drought tolerance have been reported in various studies. Many earlier studies have identified genomic regions associated with different physiological traits such as carbon isotope discrimination (ΔC) on chromosomes 1BL, 2BS, 3BS, 4AS, 4BS, 5AS, 7AS, and 7BS (Rebetzke et al., 2008); flag leaf senescence on chromosomes 2B and 2D (Verma et al., 2004); coleoptile length on chromosomes 4B and 6A (Rebetzke et al., 2001); seedling vigor on chromosome 6A (Spielmeyer et al., 2007); and canopy temperature on 1B, 2B, 3B, 4A, and 5A (Pinto et al., 2010). Building upon these earlier discoveries, the last few years have shown significant advances. For example, Tura et al. (2020) mapped yield QTLs under drought in a double haploid population on chromosomes 4A, 5B, and 7A. In a comprehensive GWAS study involving a panel of 210 European elite wheat lines, Touzy et al. (2019) discovered 24, 31, and 28 QTLs associated with drought tolerance, respectively, under low, medium and high water stress conditions. Bhatta et al. (2018) identified 90 marker-trait associations (MTAs) related to yield and associated traits under limited water conditions in a GWAS study with GBS markers using 123 synthetic hexaploid wheat lines. Similarly, QTLs for drought-related traits such as the drought susceptibility index (DSI), normalized difference vegetative index (NDVI), and leaf traits (including green leaf area, leaf senescence, and flag leaf phenotypes) were detected on chromosomes 1B, 4A, 6B, 5B, 7A, and 7B (Edae et al., 2014) in a GWAS involving a spring wheat panel. **Table 2** summarizes QTLs reported for drought tolerance related traits on different chromosomes of the wheat genome, published in the peer reviewed literature from 2011 onwards.

A number of the QTLs reported in **Table 2** that are associated with different physio-morphological traits that contribute to wheat grain yield under drought, appear to be restricted to specific genetic backgrounds and environments (Griffiths et al., 2012; Acuna-Galindo et al., 2015). To determine which QTLs are relevant across genetic backgrounds and environments, some studies have used a meta-analysis approach to identify common QTLs discovered in different studies. For example, in a QTL meta-analysis recently conducted by Soriano and Alvaro (2019), 634 QTLs retrieved from earlier studies were projected on a consensus map having 7352 markers, which resulted in 94 consensus QTL regions, of which 35 were associated with root architecture and response to moisture stress. Similarly, in another QTL meta-analysis, Acuna-Galindo et al. (2015) reported 43 meta-QTLs related to drought and heat stress of which 20 were specific to drought stress tolerance. These results suggest that QTL meta-analysis, along with better estimations of QTL effects, may maximize the benefits of past drought QTL studies (Acuna-Galindo et al., 2015; Gupta et al., 2017; Soriano and Alvaro (2019).

Once candidate genes or loci have been identified, another approach is to use allele-specific markers to identify favorable alleles (haplotypes) that promote drought tolerance in a defined population. For example, Khalid et al. (2019) in a study comprising 213 advanced lines derived from synthetic hexaploid wheat and elite cultivars (CIMMYT and Pakistan), identified favorable alleles at five candidate genes associated with drought adaptation, including those that encode a cell wall invertase gene (*TaCwi-A1*) that converts sucrose into glucose and fructose, a dehydration responsive element binding protein (*Dreb1*) and *COMT-3B* that promotes lignin under water stress. The cell wall invertase gene discovery was consistent with an earlier study involving 348 modern Chinese cultivars which identified a favorable allele (haplotype) at another invertase gene (*TaCWI-5D*) on chromosome 5D in terms of promoting drought tolerance in wheat (Jiang et al., 2015).

Combined, these advances in genome sequencing, QTL and favorable allele identification, have provided wheat breeders with numerous targets for introgression and selection of drought tolerance promoting alleles that are potentially stable across environments and genetic backgrounds.

Genome Engineering Techniques

Genome engineering techniques, including gene pyramiding, gene stacking, and transgenics, broaden the methods available to breed wheat for tolerance to abiotic stress including drought (Budak et al., 2015; Mwadzingeni et al., 2016a). Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) is a bacterium-derived genome editing system; in this system, a DNA fragment encoding a non-coding RNA sequence is designed to target and then cleave a chromosomal DNA target of interest, within the Cas9 protein complex (Garneau et al., 2010; Budak et al., 2015; Zhang et al., 2018; Kumar et al., 2019; Okada et al., 2019; El-Mounadi et al., 2020; Shinwari et al., 2020). Recently, Wang et al. (2018) used CRISPR/Cas9 to increase grain size in wheat. Kim et al. (2018) also successfully established a

TABLE 2 | Recent peer reviewed reports (2011-current) of the important physio-morphological traits associated with drought tolerance and locations of QTLs associated with these traits in wheat.

Target trait	Chromosome	Method used for QTL identification	Type of population	Environment	Reference
Days to heading	1A, 2A, 2B, 3A, 4B, 5B, and 6D	GWAS	Diversity panel	Rainfed	Gahlaut et al., 2019
	1B, 2D, 3B, and 4A	GWAS	Diversity panel	Drought stress	Mathew et al., 2019
	2A	GWAS	Diversity panel	Drought stress	Qaseem et al., 2018
	2D and 6A	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018
Days maturity	1A, 1B, 2A, and 4B	GWAS	Diversity panel	Rainfed	Gahlaut et al., 2019
	4B and 7A	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019
	2B, 5A, and 6B	GWAS	Diversity panel	Drought stress	Mathew et al., 2019
	7A	GWAS	Diversity panel	Drought stress	Qaseem et al., 2018
	1B	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018
Grain yield	4A, 3B, and 7A	Linkage mapping	Doubled haploid population	Rainfed	Gahlaut et al., 2017
	1A, 1B, 2D, and 3B	Linkage mapping	RIL population	Terminal drought stress	Zandipour et al., 2020
	1D, 3D, and 5B	GWAS	Diversity panel of 94 German winter wheat	Drought stress	Lehnert et al., 2018
	2B, 6D	GWAS	Diversity panel	Rainfed	Li L. et al., 2019
	3D, 6A, 6D, and 7B	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019
	6D	Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019
	2A and 4A	GWAS	Diversity panel	Drought stress	Mathew et al., 2019
	2A and 7A	GWAS	Diversity panel	Drought stress	Qaseem et al., 2018
	2D	GWAS	Wheat association mapping initiative (WAMI) panel	Drought stress	Lopes et al., 2015b
	1AL	GWAS	Spring wheat diversity panel	Rainfed	Ain et al., 2015
Yield stability index	2B, 3B, 4A, 5D, 6B, and 7A	GWAS	Diversity panel	Irrigated	Gahlaut et al., 2019
	1B, 2A, 2B, 3A, 3B, 5A, 5D, 6A, and 6D	GWAS	Diversity panel	Rainfed and irrigated	Ballesta et al., 2020
Harvest index	7B and 7D	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019
Spike length	1B, 3B, 4A, 5B, and 6B	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018
	1B, 2B, 2D, 3A, 4B, 5B, 6A, 6B, and 7A	GWAS	Diversity panel	Drought stress	Mwadzingeni et al., 2017
	6A	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018
Spikelet per spike	4B and 6B	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019
	6B, 2D, 2B, 5D, 1B, and 4B	GWAS	Diversity panel	Drought stress	Mwadzingeni et al., 2017
Number of kernels per spike	2B and 4B	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019
	2D and 4A	GWAS	Diversity panel	Drought stress	Mwadzingeni et al., 2017
	6A, 7A, 1B, 3B3, 3B, 6B2, 7B, and 1D	Linkage mapping	RIL population	Terminal drought stress	Zandipour et al., 2020
	2A	Linkage mapping	Doubled haploid population	Osmotic stress	Dolferus et al., 2019
	5A	Linkage mapping	Doubled haploid population	No osmotic stress	Dolferus et al., 2019
	1A, 2B, 3D, 4A, and 4B	GWAS	Diversity panel	Rainfed	Li L. et al., 2019
	2A	Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019
	6A and 7B	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018
Thousand grain weight	7A	Linkage mapping	Doubled haploid population	Rainfed	Gahlaut et al., 2017

(Continued)

TABLE 2 | Continued

Target trait	Chromosome	Method used for QTL identification	Type of population	Environment	Reference	
Grain weight per plant	6D	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018	
	7A	Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019	
	2D and 6A	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018	
	1B, 2B1, 2B2, and 3B	Linkage mapping	RIL population	Terminal drought stress	Zandipour et al., 2020	
	2A, 5A, and 6B	GWAS	Diversity panel	Rainfed	Li L. et al., 2019	
	2A	Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019	
	5A	Linkage mapping	Double haploid population	Drought stress at flowering	Fatima et al., 2018	
	1B, 2D, 3A, 3B, 3D, 4D, 5A, 6A, 6B, 7A, and 7B	Linkage mapping	Doubled haploid population	Rainfed and irrigated	Shi S. et al., 2017	
	2A	Linkage mapping	Core collection	Rainfed and irrigated	Ahmad et al., 2014	
	2A, 2D, 3A, 4A, 4B, 5B, and 6A	GWAS	Diversity panel	Rainfed	Gahlaut et al., 2019	
	1AL, 4AL, 7AS, 7DS	Linkage mapping	F ₂ :F ₃ population	Drought stressed	Nezhad et al., 2012	
	2D	GWAS	Diversity panel	Drought stress	Schmidt et al., 2020	
	1D, 2A, 2B, 3B, 4A, 4B, 4D, 5A, 5B, 5D, 6B, and 7A	GWAS	Synthetic hexaploid wheat	Drought stress	Bhatta et al., 2018	
	7D	GWAS	Diversity panel	Drought stress	Qaseem et al., 2018	
	4B and 4D	GWAS	Diversity panel	Drought stress	Schmidt et al., 2020	
	2A	GWAS	Diversity panel	Drought stress	Qaseem et al., 2018	
	5BL and 6AL	GWAS	Diversity panel	Rainfed	Ain et al., 2015	
	Awn length	5A	Linkage mapping	Double haploid population	Rainfed	Gahlaut et al., 2017
		1A and 2D	GWAS	Diversity panel	Drought stress	Mwadzingeni et al., 2017
4B		GWAS	Wheat association mapping initiative (WAMI) panel	Drought stress	Lopes et al., 2015b	
4B, 6B		GWAS	Diversity panel	Irrigated	Gahlaut et al., 2019	
1B, 3B, 5A		GWAS	Diversity panel	Rainfed	Gahlaut et al., 2019	
7B and 7D		GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
1B, 2B, 4B, 7B		GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
1B, 2A, 2B, 2D, 3A, 3B, 4B, 4D, 5A, 6B, 6D, and 7A		Linkage mapping	Double haploid population	Rainfed and irrigated	Shi S. et al., 2017	
1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 6A, 6B, 6D, 7A, and 7B		Linkage mapping	Double haploid population	Rainfed and irrigated	Shi S. et al., 2017	
2A		Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019	
6D		Linkage mapping	Synthetic derived RIL population	Drought stress	Liu et al., 2019	
1B, 2B, and 3B		Linkage mapping	BC ₂ F ₂ families	Drought stress	Barakat et al., 2015	
1B, 4A, 6B, 5B, 7A, and 7B		GWAS	Diversity panel	Drought stress	Edae et al., 2014	
7B and 7D		GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
4B and 6A		GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
7A, 7B, and 7D		GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
5A, 3B, 4B, and 6B		Linkage mapping	Mapping population	Drought stress	Czyczęło-Mysza et al., 2014	
Flag leaf senescence		5D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013
		2D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013
	1B, 2B, 2D, 3B, 5B, 7A, and 7D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	1B, 1D, 2B, 3B, 4A, and 4D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	3A, 3B, 3D	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018	
	4A	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
Flag leaf length	7B and 7D	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
	4B and 6A	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
	7A, 7B, and 7D	GWAS	Elite wheat genotypes	Drought stress	Qaseem et al., 2019	
	5A, 3B, 4B, and 6B	Linkage mapping	Mapping population	Drought stress	Czyczęło-Mysza et al., 2014	
	5D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	2D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
Flag leaf width	1B, 2B, 2D, 3B, 5B, 7A, and 7D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	1B, 1D, 2B, 3B, 4A, and 4D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	3A, 3B, 3D	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018	
	4A	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	5D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	2D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
Leaf area	1B, 2B, 2D, 3B, 5B, 7A, and 7D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	1B, 1D, 2B, 3B, 4A, and 4D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	3A, 3B, 3D	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018	
	4A	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	5D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	2D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
Germination ability	1B, 2B, 2D, 3B, 5B, 7A, and 7D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	1B, 1D, 2B, 3B, 4A, and 4D	GWAS	Diversity panel	Drought stress	Mathew et al., 2019	
	3A, 3B, 3D	GWAS	Diversity panel	Drought stress	Lehnert et al., 2018	
	4A	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	5D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	
	2D	Linkage mapping	RIL population	Osmotic stress	Zhang et al., 2013	

(Continued)

TABLE 2 | Continued

Target trait	Chromosome	Method used for QTL identification	Type of population	Environment	Reference
	2B and 3B	GWAS	Core winter wheat collection	PEG induced water stress	Ayalew et al., 2018
	3B, 4A, and 5B 5B and 5D	GWAS Linkage mapping	Core winter wheat collection Mapping population	Non-stress Drought stress	Ayalew et al., 2018 Czyżyło-Mysza et al., 2014
Root to shoot dry weight ratio	2D 2D, 5A, and 3B	GWAS Linkage mapping	A population of elite wheat varieties RIL population	Drought stress Irrigated and Osmotic stress	Ahmad et al., 2017 Zhang et al., 2013
Photosynthetic rate	2B, 4A, 4D, and 6A	GWAS	Diversity panel	Drought stress	Mathew et al., 2019
Relative water content	3A	Linkage mapping	F ₂ population	Drought stress	Maqsood et al., 2017
Cell membrane thermostability	4D	Linkage mapping	F ₂ population	Drought stress	Maqsood et al., 2017
	2B	Linkage mapping	F ₂ population	Drought stress	Maqsood et al., 2017
Abscisic acid content	2B and 6B	Linkage mapping	BC ₂ F ₂ families	Drought stress	Barakat et al., 2015
Waxiness	2A	Linkage mapping	BC ₂ F ₂ families	Drought stress	Barakat et al., 2015
Low excised-leaf water loss (ELWL)	3A, 3B, 4B, 5B, 5D, 6B, 7A, 7B, and 7D	Linkage mapping	Synthetic derived RIL population Double haploid hexaploid wheat	Drought stress Irrigated	Liu et al., 2019 Czyżyło-Mysza et al., 2018
Above ground dry weight	7B and 7D	GWAS	Elite wheat genotypes	Drought stress	Gaseem et al., 2019
Above ground biomass	1D, 2A, and 4A,	GWAS	Diversity panel of 94 German winter wheat	Drought stress	Lehnert et al., 2018

CRISPR/Cas9 genome editing system in wheat protoplasts to edit the stress-responsive factor genes *TaDREB2* and *TaERF3*. Since some essential regulatory genes that control the biosynthesis of metabolites associated with drought tolerance have been identified (Yang et al., 2010), the CRISPR/Cas9 gene editing technology could potentially be used to target such genes and traits in the future (Singh B. et al., 2018).

In addition, CRISPR/Cas9 mediated base editing (Zong et al., 2017; Li C. et al., 2018) and prime editing (Anzalone et al., 2019) techniques could be potentially utilized in breeding for drought tolerant wheat in the future as alternatives to the standard CRISPR/Cas9 genome editing system. Gene editing using CRISPR technologies, which depends on double strand breaks induced by the Cas9 protein (Garneau et al., 2010), has limitations associated with the delivery of donor DNA to the targeted cell types due to the low frequency of homologous recombination in plants (Molla and Yang, 2019; Hassan et al., 2020). The advantage of base editing technology over the standard CRISPR technology is: it does not require double stranded breaks and can simply perform substitution mutations allowing four kinds of modifications (C/G-to-T/A and A/T-to-G/C) (Komor et al., 2016; Gaudelli et al., 2017; Li C. et al., 2018; Molla and Yang, 2019). However, as base editing does not create indels (Komor et al., 2016; Gaudelli et al., 2017), an alternative prime editing technology which also does not require double stranded DNA cleavage, has been engineered to introduce indels; it also allows all 12 base to base conversions (Anzalone et al., 2019). As the prime editing method overcomes the above-stated limitations and enables different mutations (point mutations and indels) in wheat protoplasts (Lin et al., 2020), it may be a game changer technology in the future in terms of engineering drought tolerant wheat (Hassan et al., 2020; Marzec and Hensel, 2020).

In addition to gene editing, there is evidence suggesting that wheat genetic improvement is also possible through genetic engineering. For instance, transfer into wheat of the *AISAP* gene encoding a stress-associated protein from the halophyte grass/Mediterranean saltgrass (*Aeluropus litoralis*) has been shown to enhance the germination rate, biomass, and grain yield of wheat under osmotic- and salinity stress (Ben-Saad et al., 2012). A recent study (Ayadi et al., 2019) demonstrated that overexpression of the wheat aquaporin gene *TdPIP2* in transgenic wheat improved drought tolerance. Similarly, the overexpression of the *Escherichia coli CspA* gene (modified as *SeCspA*) in transgenic wheat lowered the rate of water loss and maintained higher chlorophyll and proline under drought compared to non-transgenic plants (Yu et al., 2017). Additionally, improved drought tolerance (enhanced growth, delayed senescence, higher relative water content, higher photosynthetic rate, and higher total chlorophyll content) was observed in transgenic wheat in which an *Arabidopsis thaliana* cysteine protease (OVERLY TOLERANT TO SALT-1, *OTS1*) was over-expressed (using a ubiquitin promoter) compared to non-transformed wheat plants (le Roux et al., 2019). It has also been reported that overexpression of a fructan exohydrolase encoding gene (*1-FEH w3*) promotes higher fructan remobilization under drought which contributes positively to grain yield (Zhang et al., 2015; Hou et al., 2018).

Several other studies have shown that overexpression of different drought responsive genes confers greater drought tolerance in transgenic wheat including the genes *AtHDG11* (Li et al., 2016), *TaCIPK23* (Cui et al., 2018), *TaBZR2* (Cui et al., 2019), *TaWRKY2* (Gao et al., 2018), *TaWRKY1*, and *TaWRKY33* (He et al., 2016), and *TaPYLA* (Mega et al., 2019). Interestingly, *TaSnRK2.9*, which is a sucrose non-fermenting 1-related protein kinase gene cloned from bread wheat, elevated ABA content in tobacco (*Nicotiana tabacum*) resulting in greater drought tolerance (Feng et al., 2019).

Therefore, both gene editing and genetic engineering show promise in terms of achieving drought tolerance in wheat. However, time, effort, skill, financial resources, regulatory issues, and public acceptance are major constraints to wider adoption of these techniques in wheat research programs.

Future Perspectives

The world is currently facing the prospect of food insecurity due to increasing drought. An assessment of genetic gain over fifty years of wheat breeding at CIMMYT showed an increase in grain yield by ~18 kg/ha per year under drought (Mondal et al., 2020) but this rate will be insufficient to meet demand. As this review has shown, promising approaches to improve drought tolerance in wheat include exploration of wheat diversity in genebanks, identification of genome-wide QTLs, validation of putative QTLs, and introgression of markers, genomic segments or candidate genes. However, undertaking this pipeline *de novo* is a major challenge in terms of time, skill, and resources. Therefore, the QTL meta-analysis approach (Acuna-Galindo et al., 2015; Gupta et al., 2017; Soriano and Alvaro, 2019) could be an immediate option that utilizes the results of already-existing QTLs associated with drought tolerance in wheat. Another promising near-term technique is genome editing using CRISPR/Cas9, given the potential it has shown to improve traits such as male sterility, grain weight, protein content, and powdery mildew resistance (Zhang Y. et al., 2017; Wang et al., 2018; Zhang et al., 2018; Okada et al., 2019). Furthermore, the pioneering CRISPR-based technologies including base editing and prime editing may have potential future. Though transgenic wheat offers potential, public acceptance may limit its adoption. By contrast, synthetic hexaploid wheats (SHWs) have shown promise in terms of drought tolerance and simplify the use of existing wild genetic resources (Lopes and Reynolds, 2011; Sohail et al., 2011; Becker et al., 2016; Aberkane et al., 2019; Laxa et al., 2019).

Apart from the use of wider germplasm, the growth environment in which selection and breeding is undertaken is important as well as the phenotyping strategies employed. The tradition of breeding for drought tolerance in wheat has been to compare grain yield in a water deficit environment compared to an optimal environment (Khakwani et al., 2011). However, the complex nature of drought (e.g., drought on sandy soil is more severe than on clay soil) complicates these efforts in terms of their actual impact on yield stability (Hoover et al., 2018). Furthermore, the large and complex nature of the wheat genome makes breeding for drought-tolerant wheat

challenging. Therefore, adoption of a comprehensive strategy to develop drought-tolerant wheat varieties is needed. Such a strategy requires precise phenotyping in a water deficit environment (Fleury et al., 2010) as the efficiency of genomics also rests on advances in phenomics (Araus and Cairns, 2014; Fahlgren et al., 2015; Bai et al., 2016; Fernandez et al., 2017; Zhang X. et al., 2017). Many ground-based and aerial phenotyping platforms have been developed recently, with advances made in remote sensing, aeronautics, and computing (White et al., 2012; Araus and Cairns, 2014). In addition, new bioinformatics platforms have shown potential to overcome challenges associated with the management of the high volume of data generated by precision phenotyping tools (Shi et al., 2016; Singh et al., 2016; Coppens et al., 2017; Araus et al., 2018; Yang et al., 2020). These include Minimum Information About a Plant Phenotyping Experiment (MIAPPE), a platform that enables harmonization of phenotyping experiments (Bolger et al., 2019); and Crop Ontology, a platform that promotes proper use of genotypic and phenotypic data through data annotation (Shrestha et al., 2012).

Global agricultural research institutions such as CIMMYT and ICARDA have already initiated precision phenotyping coupled with NGS protocols to improve the utilization of wheat genebank materials and their development into pre-breeding materials for stress tolerance breeding programs (Crossa et al., 2016; Singh S. et al., 2018; Singh et al., 2019). However, other national and international institutions holding wheat genetic materials may need to prioritize similar efforts in order to deploy potential germplasm into wheat breeding programs. Traditional efforts may be revived to generate new allelic diversity for drought tolerance traits through mutagenesis involving chemical mutagens, ultraviolet light, and high energy radiation (Chen et al., 2014). Similarly, to discover the genetic basis of complex traits, transcriptomics, proteomics, and metabolomics offer promise (Reddy et al., 2014). For example, the information generated concerning different metabolic pathways have potential to add value in terms of developing targeted strategies that modulate the expression of genes associated with stress tolerance (Yang et al., 2010).

Further enhancement and strengthening of current global research collaborations is another way forward. CIMMYT is using the concept of 12 mega environments (ME) (<http://wheatatlas.org/megaenvironments>), defined as broad geographic regions with similar biotic and abiotic stresses, as well as agronomic practices and consumer preferences, to develop and promote wheat cultivars for wider adoption. One immediate activity of this collaboration could be to support the initiation of genomic characterization of genebank materials in developing countries. In the same vein, a very promising global initiative is the “The Heat and Drought Wheat Improvement Consortium (HeDWIC)” (<https://www.hedwic.org>), which is a worldwide platform of wheat scientists from more than 90 countries working on drought and heat tolerance. Similarly, “The International Wheat Yield Partnership (IWYP)” (<https://iwyp.org>) is a recent global consortium of partners including public and private research organizations, that aims to improve the genetic potential of

wheat grain yield by 50% through collaborative efforts in two decades. It is hoped that such global scientific cooperation in wheat breeding has the potential to generate new drought-tolerant varieties to combat climate change.

AUTHOR CONTRIBUTIONS

KK and AN conceptualized the manuscript, while KK undertook the literature review, analysis, and wrote the manuscript, and AN and MR edited the manuscript.

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REFERENCES

- Abdolshahi, R., Safarian, A., Nazari, M., Pourseyedi, S., and Mohamadi-Nejad, G. (2013). Screening drought-tolerant genotypes in bread wheat (*Triticum aestivum* L.) using different multivariate methods. *Arch. Agron. Soil Sci.* 59, 685–704. doi: 10.1080/03650340.2012.667080
- Aberkane, H., Payne, T., Kishi, M., Amri, A., and Jamora, N. (2019). *Reaching into the past to tackle new challenges: Improving wheat by conserving wild "goat grass"*. *Genebank Impacts Brief No. 2. CGIAR Genebank Platform* (Bonn (Germany): Crop Trust). Available at: <https://repository.cimmyt.org/bitstream/handle/10883/20626/61255.pdf?sequence=1&isAllowed=y>.
- Abid, M., Tian, Z., Ata-ul-karim, S. T., Liu, Y., Cui, Y., Zahoor, R., et al. (2016). Improved tolerance to post-anthesis drought stress by pre-drought priming at vegetative stages in drought-tolerant and -sensitive wheat cultivars. *Plant Physiol. Biochem.* 106, 218–227. doi: 10.1016/j.plaphy.2016.05.003
- Abido, W. A. E., and Zsombik, L. (2018). Effect of water stress on germination of some Hungarian wheat landraces varieties. *Acta Ecol. Sin.* 38, 422–428. doi: 10.1016/j.chnaes.2018.03.004
- Abrouk, M., Stritt, C., Muller, T., Keller, B., Roulin, A. C., and Krattinger, S. G. (2018). High-throughput genotyping of the spelt gene pool reveals patterns of agricultural history in Europe. *bioRxiv* 481424. doi: 10.1101/481424
- Acuna-Galindo, M. A., Mason, R. E., Subramanian, N. K., and Hays, D. B. (2015). Meta-analysis of wheat QTL regions associated with adaptation to drought and heat stress. *Crop Sci.* 55, 477–492. doi: 10.2135/cropsci2013.11.0793
- Ahmad, M. Q., Khan, S. H., Khan, A. S., Kazi, A. M., and Basra, S. M. A. (2014). Identification of QTLs for drought tolerance traits on wheat chromosome 2A using association mapping. *Int. J. Agric. Biol.* 16, 862–870.
- Ahmad, I., Ali, N., Ahmad, H., and Innamullah, (2017). "Association mapping of root traits for drought tolerance in bread wheat," in *Wheat Improvement, Management and Utilization*. Eds. R. Wanyera and J. Owuochi (London: INTECH), 39–57. doi: 10.5772/67242
- Ahmadi, J., Pour-Aboughadareh, A., Fabriki-Ourang, S., Mehrabi, A. A., and Siddique, K. H. M. (2018). Screening wheat germplasm for seedling root architectural traits under contrasting water regimes: potential sources of variability for drought adaptation. *Arch. Agron. Soil Sci.* 64, 1351–1365. doi: 10.1080/03650340.2018.1432855
- Ahmed, H. G. M. D., Sajjad, M., Li, M., Azmat, M. A., Rizwan, M., Maqsood, R. H., et al. (2019). Selection criteria for drought-tolerant bread wheat genotypes at seedling stage. *Sustain* 11, 1–17. doi: 10.3390/su11092584
- Ain, Q., Rasheed, A., Anwar, A., Mahmood, T., Imtiaz, M., Mahmood, T., et al. (2015). Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. *Front. Plant Sci.* 6, 743. doi: 10.3389/fpls.2015.00743

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- Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., et al. (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature* 576, 149–157. doi: 10.1038/s41586-019-1711-4
- Araus, J. L., and Cairns, J. E. (2014). Field high-throughput phenotyping: The new crop breeding frontier. *Trends Plant Sci.* 19, 52–61. doi: 10.1016/j.tplants.2013.09.008
- Araus, J. L., Kefauver, S. C., Zaman-Allah, M., Olsen, M. S., and Cairns, J. E. (2018). Translating high-throughput phenotyping into genetic gain. *Trends Plant Sci.* 23, 451–466. doi: 10.1016/j.tplants.2018.02.001
- Arshad, Y., Zahravi, M., and Hassanpour, J. (2016). Identification of sources for drought tolerance in local bread wheat landraces. *Iran. J. Genet. Plant Breed.* 5, 22–31.
- Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.* 28, 169–183. doi: 10.1016/j.biotechadv.2009.11.005
- Ayadi, M., Brini, F., and Masmoudi, K. (2019). Overexpression of a wheat aquaporin gene, *tdpjp2;1*, enhances salt and drought tolerance in transgenic durum wheat cv. Maali. *Int. J. Mol. Sci.* 20:2389. doi: 10.3390/ijms20102389
- Ayalew, H., Liu, H., Börner, A., Kobiljski, B., Liu, C., and Yan, G. (2018). Genome-wide association mapping of major root length QTLs under PEG induced water stress in wheat. *Front. Plant Sci.* 871, 1759. doi: 10.3389/fpls.2018.01759
- Bai, G., Ge, Y., Hussain, W., Baenziger, P. S., and Graef, G. (2016). A multi-sensor system for high throughput field phenotyping in soybean and wheat breeding. *Comput. Electron. Agric.* 128, 181–192. doi: 10.1016/j.compag.2016.08.021
- Ballesta, P., Mora, F., and Del Pozo, A. (2020). Association mapping of drought tolerance indices in wheat: QTL-rich regions on chromosome 4A. *Sci. Agric.* 77, e20180153. doi: 10.1590/1678-992X-2018-0153
- Barakat, M. N., Moustafa, K. A., Elshafei, A. A., and Salem, A. K. (2015). Identification of QTLs for four physiological traits in an advanced backcross population of wheat under drought stress. *Plant Omics J.* 8, 122–129.
- Becker, S. R., Byrne, P. F., Reid, S. D., Bauerle, W. L., McKay, J. K., and Haley, S. D. (2016). Root traits contributing to drought tolerance of synthetic hexaploid wheat in a greenhouse study. *Euphytica* 207, 213–224. doi: 10.1007/s10681-015-1574-1
- Ben-Saad, R., Ben-Ramdhan, W., Zouari, N., Azaza, J., Mieulet, D., Guiderdoni, E., et al. (2012). Marker-free transgenic durum wheat cv. Karim expressing the ALSAP gene exhibits a high level of tolerance to salinity and dehydration stresses. *Mol. Breed.* 30, 521–533. doi: 10.1007/s11032-011-9641-3
- Bhatta, M., Morgounov, A., Belamkar, V., and Baenziger, P. S. (2018). Genome-wide association study reveals novel genomic regions for grain yield and yield-related traits in drought-stressed synthetic hexaploid wheat. *Int. J. Mol. Sci.* 19, 3011. doi: 10.3390/ijms19103011
- Bi, H., Kovalchuk, N., Langridge, P., Tricker, P. J., Lopato, S., and Borisjuk, N. (2017). The impact of drought on wheat leaf cuticle properties. *BMC Plant Biol.* 17, 85. doi: 10.1186/s12870-017-1033-3

- Bolger, A. M., Poorter, H., Dumschott, K., Bolger, M. E., Arend, D., Osorio, S., et al. (2019). Computational aspects underlying genome to phenome analysis in plants. *Plant J.* 97, 182–198. doi: 10.1111/tj.14179
- Budak, H., Hussain, B., Khan, Z., Ozturk, N. Z., and Ullah, N. (2015). From genetics to functional genomics: Improvement in drought signaling and tolerance in wheat. *Front. Plant Sci.* 6:1012:1012. doi: 10.3389/fpls.2015.01012
- Cai, X., and Liu, D. (1989). Identification of a 1B/1R wheat-rye chromosome translocation. *Theor. Appl. Genet.* 77, 81–83. doi: 10.1007/BF00292320
- Chen, X., Min, D., Ahmad, T., and Hu, Y. (2012). Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). *Field Crop Res.* 137, 195–201. doi: 10.1016/j.fcr.2012.09.008
- Chen, L., Hao, L., Parry, M. A. J., Phillips, A. L., and Hu, Y. G. (2014). Progress in TILLING as a tool for functional genomics and improvement of crops. *J. Integr. Plant Biol.* 56, 425–443. doi: 10.1111/jipb.12192
- Choudhary, M., Wani, S. H., Kumar, P., Bagaria, P. K., Rakshit, S., Roorkiwal, M., et al. (2019). QTLian breeding for climate resilience in cereals: progress and prospects. *Funct. Integr. Genomics* 19, 685–701. doi: 10.1007/s10142-019-00684-1
- Chung, Y. S., Choi, S. C., Jun, T. H., and Kim, C. (2017). Genotyping-by-sequencing: A promising tool for plant genetics research and breeding. *Hortic. Environ. Biotechnol.* 58, 425–431. doi: 10.1007/s13580-017-0297-8
- Coppens, F., Wuyts, N., Inzé, D., and Dhondt, S. (2017). Unlocking the potential of plant phenotyping data through integration and data-driven approaches. *Curr. Opin. Syst. Biol.* 4, 58–63. doi: 10.1016/j.coisb.2017.07.002
- Corvin, A., Craddock, N., and Sullivan, P. F. (2014). Genome-wide association studies: A primer. *Psychol. Med.* 40, 1063–1077. doi: 10.1017/S0033291709991723
- Crespo-Herrera, L. A., Crossa, J., Huerta-Espino, J., Vargas, M., Mondal, S., Velu, G., et al. (2018). Genetic gains for grain yield in CIMMYT's semi-arid wheat yield trials grown in subtropical environments. *Crop Sci.* 58, 1890–1898. doi: 10.2135/cropsci2018.01.0017
- Crossa, J., Jarquín, D., Franco, J., Pérez-Rodríguez, P., Burgueño, J., Saint-Pierre, C., et al. (2016). Genomic prediction of gene bank wheat landraces. *G3 Genes Genom. Genet.* 6, 1819–1834. doi: 10.1534/g3.116.029637
- Cui, X., Du, Y., Fu, J., Yu, T., Wang, C., Chen, M., et al. (2018). Wheat CBL-interacting protein kinase 23 positively regulates drought stress and ABA responses. *BMC Plant Biol.* 18, 93. doi: 10.1186/s12870-018-1306-5RESEARCH
- Cui, X.-Y., Gao, Y., Guo, J., Yu, T.-F., Zheng, W.-J., Liu, Y.-W., et al. (2019). BES / BZR transcription factor TaBZR2 positively regulates drought responses by activation of TaGST1. *Plant Physiol.* 180, 605–620. doi: 10.1104/pp.19.00100
- Czyczyło-Mysza, I., Marcińska, I., Skrzypek, E., Cyganek, K., Juzoń, K., and Karbarz, M. (2014). QTL mapping for germination of seeds obtained from previous wheat generation under drought. *Cent. Eur. J. Biol.* 9, 374–382. doi: 10.2478/s11535-013-0273-y
- Czyczyło-Mysza, I. M., Marcińska, I., Skrzypek, E., Bocianowski, J., Dziurka, K., Rančić, D., et al. (2018). Genetic analysis of water loss of excised leaves associated with drought tolerance in wheat. *PeerJ* 6, e5063. doi: 10.7717/peerj.5063
- Daryanto, S., Wang, L., and Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PLoS One* 11, e0156362. doi: 10.1371/journal.pone.0156362
- Dashti, H., Yazdi-Samadi, B., Ghannadha, M., Naghavi, M., and Quarri, S. (2007). QTL analysis for drought resistance in wheat using doubled haploid lines. *Int. J. Agric. Biol.* 9, 98–102.
- Ding, J., Huang, Z., Zhu, M., Li, C., Zhu, X., and Guo, W. (2018). Does cyclic water stress damage wheat yield more than a single stress? *PLoS One* 13, e0195535. doi: 10.1371/journal.pone.0195535
- Dolferus, R., Thavamani Kumar, S., Sangma, H., Kleven, S., Wallace, X., Forrest, K., et al. (2019). Determining the genetic architecture of reproductive stage drought tolerance in wheat using a correlated trait and correlated marker effect model. *G3 Genes Genom. Genet.* 9, 473–489. doi: 10.1534/g3.118.200835
- Dreisigacker, S., Kishii, M., Lage, J., and Warburton, M. (2008). Use of synthetic hexaploid wheat to increase diversity for CIMMYT bread wheat improvement. *Aust. J. Agric. Res.* 59, 413–420. doi: 10.1071/AR072250004-9409/08/050413
- Eade, E., Byrne, P. F., Haley, S. D., Lopes, M. S., and Reynolds, M. P. (2014). Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. *Theor. Appl. Genet.* 127, 791–807. doi: 10.1007/s00122-013-2257-8
- El-Mounadi, K., Morales-Floriano, M. L., and Garcia-Ruiz, H. (2020). Principles, applications, and biosafety of plant genome editing using CRISPR-Cas9. *Front. Plant Sci.* 11, 56. doi: 10.3389/fpls.2020.00056
- Fahlgren, N., Gehan, M. A., and Baxter, I. (2015). Lights, camera, action: High-throughput plant phenotyping is ready for a close-up. *Curr. Opin. Plant Biol.* 24, 93–99. doi: 10.1016/j.pbi.2015.02.006
- FAO (1998). *The state of the world's plant genetic resources for food and agriculture* (Rome, Italy: Food and Agriculture Organization of the United Nations). Available at: <http://www.fao.org/3/a-w7324e.pdf>.
- FAO (2018). *Crop prospects and food situation. Quarterly global report* (Rome, Italy: Food and Agriculture Organization of the United Nations). Available at: <http://www.fao.org/3/i8764en/I8764EN.pdf>.
- Farshadfar, E., Saedi, M., and Honarmand, S. J. (2012). Evaluation of drought tolerance screening techniques among some landraces of bread wheat genotypes. *Eur. J. Exp. Biol.* 2, 1585–1592.
- Fatima, S., Chaudhari, S. K., Akhtar, S., Amjad, M. S., Akbar, M., Iqbal, M. S., et al. (2018). Mapping QTLs for yield and yield components under drought stress in bread wheat (*Triticum aestivum* L.). *Appl. Ecol. Environ. Res.* 16, 4431–4453. doi: 10.15666/aer/1604_44314453
- Feng, F., Wang, L., Wu, Y., Luo, Q., Zhang, Y., Qui, D., et al. (2019). TaSnRK2.9, a sucrose non-fermenting 1-related protein kinase gene, positively regulates plant response to drought and salt stress in transgenic tobacco. *Front. Plant Sci.* 9, 2003. doi: 10.3389/fpls.2018.02003
- Fernandez, M. G. S., Bao, Y., Tang, L., and Schnable, P. S. (2017). A high-throughput, field-based phenotyping technology for tall biomass crops. *Plant Physiol.* 174, 2008–2022. doi: 10.1104/pp.17.00707
- Fleury, D., Jefferies, S., Kuchel, H., and Langridge, P. (2010). Genetic and genomic tools to improve drought tolerance in wheat. *J. Exp. Bot.* 61, 3211–3222. doi: 10.1093/jxb/erq152
- Gahlaut, V., Jaiswal, V., Tyagi, B. S., Singh, G., Sareen, S., Balyan, H. S., et al. (2017). QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. *PLoS One* 12, e0182857. doi: 10.1371/journal.pone.0182857
- Gahlaut, V., Jaiswal, V., Singh, S., Balyan, H. S., and Gupta, P. K. (2019). Multi-locus genome wide association mapping for yield and its contributing traits in hexaploid wheat under different water regimes. *Sci. Rep.* 9, 19486. doi: 10.1038/s41598-019-55520-0
- Gao, H., Wang, Y., Xu, P., and Zhang, Z. (2018). Overexpression of a WRKY transcription factor TaWRKY2 enhances drought stress tolerance in transgenic wheat. *Front. Plant Sci.* 9, 997. doi: 10.3389/fpls.2018.00997
- Garneau, J. E., Dupuis, M.-E., Villion, M., Romero, D., Barrangou, R., Boyaval, P., et al. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 468, 67–72. doi: 10.1038/nature09523
- Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. II, et al. (2017). Programmable base editing of A.T to G.C in genomic DNA without DNA cleavage. *Nature* 551, 464–471. doi: 10.1038/nature24644
- GCARD (2012). Breakout session P1.1 National Food Security – The Wheat Initiative – an International Research Initiative for Wheat Improvement. *Second Global Conference on Agricultural Research for Development*, Punta del Este, Uruguay (29 Oct–1 Nov, 2012). Available at: [http://www.fao.org/docs/eims/upload/306175/Briefing Paper \(3\)-Wheat Initiative - Hélène Lucas.pdf](http://www.fao.org/docs/eims/upload/306175/Briefing Paper (3)-Wheat Initiative - Hélène Lucas.pdf).
- Griffiths, S., Simmonds, J., Leverington, M., Wang, Y., Fish, L., Sayers, L., et al. (2012). Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. *Mol. Breed.* 29, 159–171. doi: 10.1007/s11032-010-9534-x
- Grover, G., Sharma, A., Gill, H. S., Srivastava, P., and Bains, N. S. (2018). *Rht8* gene as an alternate dwarfing gene in elite Indian spring wheat cultivars. *PLoS One* 13, e0199330. doi: 10.1371/journal.pone.0199330
- Guellim, A., Catterou, M., Chabrierie, O., Tetu, T., Hirel, B., Dubois, F., et al. (2019). Identification of phenotypic and physiological markers of salt stress tolerance in durum wheat (*Triticum durum* desf.) through integrated analyses. *Agronomy* 9, 844. doi: 10.3390/agronomy9120844
- Gupta, P., Balyan, H., and Gahlaut, V. (2017). QTL analysis for drought tolerance in wheat: Present status and future possibilities. *Agronomy* 7, 5. doi: 10.3390/agronomy7010005
- Gupta, P. K., Balyan, H. S., Sharma, S., and Kumar, R. (2020). Genetics of yield, abiotic stress tolerance and biofortification in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 133, 1569–1602. doi: 10.1007/s00122-020-03583-3

- Hafsi, M., Hadji, A., Guendouz, A., and Maamari, K. (2013) Relationship between flag leaf senescence and grain yield in durum wheat grown under drought conditions. *J. Agron.* 12, 69–77.
- Hassan, M., Yuan, G., Chen, J., Tuskan, G. A., and Yang, X. (2020). Review article prime editing technology and its prospects for future applications in plant biology research. *BioDesign Res.* 2020, 9350905. doi: 10.34133/2020/9350905
- Haudry, A., Cenci, A., Ravel, C., Bataillon, T., Brunel, D., Poncet, C., et al. (2007). Grinding up wheat: A massive loss of nucleotide diversity since domestication. *Mol. Biol. Evol.* 24, 1506–1517. doi: 10.1093/molbev/msm077
- He, G. H., Xu, J. Y., Wang, X. Y., Liu, J. M., Li, P. S., Chen, M., et al. (2016). Drought-responsive WRKY transcription factor genes TaWRKY1 and TaWRKY33 from wheat confer drought and/or heat resistance in *Arabidopsis*. *BMC Plant Biol.* 16, 116. doi: 10.1186/s12870-016-0806-4
- Heslop-Harrison, J. S., Leitch, A. R., Schwarzacher, T., and Anamthawat-Jonsson, K. (1990). Detection and characterization of 1B/1R translocations in hexaploid wheat. *Heredity* 65, 385–392. doi: 10.1038/hdy.1990.108
- Hoffmann, B. (2008). Alteration of drought tolerance of winter wheat caused by translocation of rye chromosome segment 1RS. *Cereal Res. Commun.* 36, 269–278. doi: 10.1556/CRC.36.2008.2.7
- HongBo, S., ZongSuo, L., MingAn, S., ShiMeng, S., and ZanMin, H. (2005). Investigation on dynamic changes of photosynthetic characteristics of 10 wheat (*Triticum aestivum* L.) genotypes during two vegetative-growth stages at water deficits. *Colloids Surf. B. Biointerf.* 43, 221–227. doi: 10.1016/j.colsurfb.2005.05.005
- Hoover, D., Wilcox, K. R., and Young, K. (2018). Experimental droughts with rainout shelters: A methodological review. *Ecosphere* 9, e02088. doi: 10.1002/ecs2.2088
- Hou, J., Huang, X., Sun, W., Du, C., Wang, C., Xie, Y., et al. (2018). Accumulation of water-soluble carbohydrates and gene expression in wheat stems correlates with drought resistance. *J. Plant Physiol.* 231, 182–191. doi: 10.1016/j.jplph.2018.09.017
- Huang, X., and Han, B. (2014). Natural variations and genome-wide association studies in crop plants. *Annu. Rev. Plant Biol.* 65, 531–551. doi: 10.1146/annurev-arplant-050213-035715
- IPCC (2013). “Summary for policymakers”, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the International Panel on Climate Change*. Eds. T. Stocker, D. Qin, G. Plattner, M. Tignor, S. Allen and J. Boschung (Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press). Available at: <https://www.ipcc.ch/report/ar5/wg1/>.
- IWGSC (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361, eaar7191. doi: 10.1126/science.aar7191
- Jafarzadeh, J., Bonnett, D., Jannink, J. L., Akdemir, D., Dreisigacker, S., and Sorrells, M. E. (2016). Breeding value of primary synthetic wheat genotypes for grain yield. *PLoS One* 11, e0162860. doi: 10.1371/journal.pone.0162860
- Janni, M., Bozzini, T., Di Giovanni, M., Moschetti, I., Lupi, R., Gennaro, A., et al. (2018). First production of wild emmer (*Triticum turgidum* ssp. *dicoccoides*) transgenic plants. *Plant Cell. Tissue Organ Cult.* 132, 461–467. doi: 10.1007/s11240-017-1342-0
- Jiang, Y., Jiang, Q., Hao, C., Hou, J., Wang, L., Zhang, H., et al. (2015). A yield associated gene TaCWI, in wheat: its function, selection and evolution in global breeding revealed by haplotype analysis. *Theor. Appl. Genet.* 128, 131–143. doi: 10.1007/s00122-014-2417-5
- Kadioglu, A., and Terzi, R. (2007). A dehydration avoidance mechanism: Leaf rolling. *Bot. Rev.* 73, 290–302. doi: 10.1663/0006-8101(2007)73[290:ADAMLR]2.0.CO;2
- Khadka, K., Earl, H., Raizada, M. N., and Navabi, A. (2020). A physiological trait-based approach for breeding drought-tolerant wheat. *Front. Plant Sci.* 11, 715. doi: 10.3389/fpls.2020.00715
- Khakwani, A. A., Dennett, M. D., and Munir, M. (2011). Drought tolerance screening of wheat varieties by inducing water stress conditions. *Songklanakarinn J. Sci. Technol.* 33, 135–142.
- Khalid, M., Afzal, F., Gul, A., Amir, R., Subhani, A., Ahmed, Z., et al. (2019). Molecular characterization of 87 functional genes in wheat diversity panel and their association with phenotypes under well-watered and water-limited conditions. *Front. Plant Sci.* 10, 717. doi: 10.3389/fpls.2019.00717
- Khan, Z., Qazi, J., Rasheed, A., and Mujeeb-Kazi, A. (2016). Diversity in D-genome synthetic hexaploid wheat association panel for seedling emergence traits under salinity stress. *Plant Genet. Resour.* 15, 488–495. doi: 10.1017/S1479262116000198
- Khayatnezhad, M., Zaeifzadeh, M., and Gholamin, R. (2011). Effect of end-season drought stress on chlorophyll fluorescence and content of antioxidant enzyme superoxide dismutase enzyme (SOD) in susceptible and tolerant genotypes of durum wheat. *Afr. J. Agric. Res.* 6, 6397–6406. doi: 10.5897/AJAR11.250
- Kilian, B., and Graner, A. (2012). NGS technologies for analyzing germplasm diversity in genebanks. *Brief. Funct. Genomics* 11, 38–50. doi: 10.1093/bfpg/elr046
- Kim, D., Alptekin, B., and Budak, H. (2018). CRISPR/Cas9 genome editing in wheat. *Funct. Integr. Genomics* 18, 31–41. doi: 10.1007/s10142-017-0572-x
- Kira, O., Linker, R., and Gitelson, A. (2015). Non-destructive estimation of foliar chlorophyll and carotenoid contents: Focus on informative spectral bands. *Int. J. Appl. Earth Obs. Geoinf.* 38, 251–260. doi: 10.1016/j.jag.2015.01.003
- Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., and Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533, 420–424. doi: 10.1038/nature17946
- Kooke, R., Kruijer, W., Bours, R., Becker, F., Kuhn, A., Geest, H., et al. (2016). Genome-wide association mapping and genomic prediction elucidate the genetic architecture of morphological traits in arabidopsis. *Plant Physiol.* 170, 2187–2203. doi: 10.1104/pp.15.00997
- Kumar, S., and Singh, B. (2009). Effect of water stress on carbon isotope discrimination and Rubisco activity in bread and durum wheat genotypes. *Physiol. Mol. Biol. Plants* 15, 281–286. doi: 10.1007/s12298-009-0032-8
- Kumar, S., Kumar, N., Balyan, H. S., and Gupta, P. K. (2003). 1BL.1RS translocation in some Indian bread wheat genotypes and strategies for its use in future wheat breeding. *Caryologia* 56, 23–30. doi: 10.1080/00087114.2003.10589303
- Kumar, R., Kaur, A., Pandey, A., Mamrutha, H. M., and Singh, G. P. (2019). CRISPR-based genome editing in wheat: a comprehensive review and future prospects. *Mol. Biol. Rep.* 46, 3557–3569. doi: 10.1007/s11033-019-04761-3
- Laxa, M., Liebthal, M., Telman, W., Chibani, K., and Dietz, K. J. (2019). The role of the plant antioxidant system in drought tolerance. *Antioxidants* 8:94. doi: 10.3390/antiox8040094
- le Roux, M. L., Kunert, K. J., van der Vyver, C., Cullis, C. A., and Botha, A. M. (2019). Expression of a small ubiquitin-like modifier protease increases drought tolerance in wheat (*Triticum aestivum* L.). *Front. Plant Sci.* 10, 266. doi: 10.3389/fpls.2019.00266
- Lehnert, H., Serfling, A., Friedt, W., and Ordon, F. (2018). Genome-wide association studies reveal genomic regions associated with the response of wheat (*Triticum aestivum* L.) to mycorrhizae under drought stress conditions. *Front. Plant Sci.* 9, 1728. doi: 10.3389/fpls.2018.01728
- Li, L., Zheng, M., Deng, G., Liang, J., Zhang, H., Pan, Z., et al. (2016). Overexpression of AtHDG11 enhanced drought tolerance in wheat (*Triticum aestivum* L.). *Mol. Breed.* 36, 23. doi: 10.1007/s11032-016-0447-1
- Li, C., Zong, Y., Wang, Y., Jin, S., Zhang, D., Song, Q., et al. (2018). Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion. *Genome Biol.* 19, 59. doi: 10.1186/s13059-018-1443-z
- Li, A., Liu, D., Yang, W., Kishii, M., and Mao, L. (2018). Synthetic hexaploid wheat: Yesterday, today and tomorrow. *Engineering* 4, 552–558. doi: 10.1016/j.eng.2018.07.001
- Li, G., Xu, X., Tan, C., Carver, B. F., Bai, G., Wang, X., et al. (2019). Identification of powdery mildew resistance loci in wheat by integrating genome-wide association study (GWAS) and linkage mapping. *Crop J.* 7, 294–306. doi: 10.1016/j.cj.2019.01.005
- Li, L., Mao, X., Wang, J., Chang, X., Reynolds, M., and Jing, R. (2019). Genetic dissection of drought and heat-responsive agronomic traits in wheat. *Plant Cell Environ.* 42, 2540–2553. doi: 10.1111/pce.13577
- Lin, Q., Zong, Y., Xue, C., Wang, S., Jin, S., Zhu, Z., et al. (2020). Prime genome editing in rice and wheat. *Nat. Biotechnol.* 38, 582–585. doi: 10.1038/s41587-020-0455-x
- Liu, C., Yang, Z., and Hu, Y. (2015). Drought resistance of wheat alien chromosome addition lines evaluated by membership function value based on multiple traits and drought resistance index of grain yield. *Field Crop Res.* 179, 103–112. doi: 10.1016/j.fcr.2015.04.016

- Liu, Y., Bowman, B., Hu, Y. G., Liang, X., Zhao, W., Wheeler, J., et al. (2017). Evaluation of agronomic traits and drought tolerance of winter wheat accessions from the USDA-ARS National Small Grains Collection. *Agronomy* 7, 51. doi: 10.3390/agronomy7030051
- Liu, K., Sun, X., Ning, T., Duan, X., Wang, Q., Liu, T., et al. (2018). Genetic dissection of wheat panicle traits using linkage analysis and a genome-wide association study. *Theor. Appl. Genet.* 131, 1073–1090. doi: 10.1007/s00122-018-3059-9
- Liu, C., Sukumaran, S., Claverie, E., Sansaloni, C., Dreisigacker, S., and Reynolds, M. (2019). Genetic dissection of heat and drought stress QTLs in phenology-controlled synthetic-derived recombinant inbred lines in spring wheat. *Mol. Breed.* 39, 34. doi: 10.1007/s11032-019-0938-y
- Lopes, M. S., and Reynolds, M. P. (2010). Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37, 147–156. doi: 10.1071/FP09121
- Lopes, M., and Reynolds, M. P. (2011). Drought adaptive traits and wide adaptation in elite lines derived from resynthesized hexaploid wheat. *Crop Sci.* 51, 1617–1621. doi: 10.2135/cropsci2010.07.0445
- Lopes, M. S., El-basyoni, I., Baenziger, P. S., Singh, S., Royo, C., Ozbek, K., et al. (2015a). Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *J. Exp. Bot.* 66, 3477–3486. doi: 10.1093/jxb/erv122
- Lopes, M. S., Dreisigacker, S., Peña, R. J., Sukumaran, S., and Reynolds, M. P. (2015b). Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. *Theor. Appl. Genet.* 128, 453–464. doi: 10.1007/s00122-014-2444-2
- Maqsood, R. H., Amjid, M. W., Saleem, M. A., Shabbir, G., and Khaliq, I. (2017). Identification of genomic regions conferring drought tolerance in bread wheat using ISSR markers. *Pakistan J. Bot.* 49, 1821–1827.
- Marcussen, T., Sandve, S. R., Heier, L., Spannagl, M., Pfeifer, M., et al. (2014). Ancient hybridizations among the ancestral genomes of bread wheat. *Science* 345, 1250092. doi: 10.1126/science.1250092
- Marzec, M., and Hensel, G. (2020). Prime editing: Game changer for modifying plant genomes trends in plant science. *Trends Plant Sci.* xx, 1–3. doi: 10.1016/j.tplants.2020.05.008
- Mathew, I., Shimelis, H., Shayanowako, A.II, Laing, M., and Chaplot, V. (2019). Genome-wide association study of drought tolerance and biomass allocation in wheat. *PLoS One* 14, e0225383. doi: 10.1371/journal.pone.0225383
- Meena, R. P., Tripathi, S. C., Chander, S., Chookar, R. S., Verma, M. A., and Sharma, R. K. (2015). Identifying drought-tolerant wheat varieties using different indices. *SAARC J. Agric.* 13, 148–161. doi: 10.3329/sja.v13i1.24188
- Mega, R., Abe, F., Kim, J. S., Tsuboi, Y., Tanaka, K., Kobayashi, H., et al. (2019). Tuning water-use efficiency and drought tolerance in wheat using abscisic acid receptors. *Nat. Plants* 5, 153–159. doi: 10.1038/s41477-019-0361-8
- Merah, O., Deléens, E., Souyris, I., and Monneveux, P. (2000). Effect of glaucousness on carbon isotope discrimination and grain yield in durum wheat. *J. Agron. Crop Sci.* 185, 259–265. doi: 10.1046/j.1439-037X.2000.00434.x
- Mia, S., Liu, H., Wang, X., and Yan, G. (2019). Multiple near-isogenic lines targeting a QTL hotspot of drought tolerance showed contrasting performance under post-anthesis water stress. *Front. Plant Sci.* 10, 271. doi: 10.3389/fpls.2019.00271
- Mickly, B. M., and Aldesuquy, H. S. (2017). Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes. *Egypt. J. Basic Appl. Sci.* 4, 47–54. doi: 10.1016/j.ejbas.2016.10.001
- Mohammadi, R. (2018). Breeding for increased drought tolerance in wheat: A review. *Crop Pasture Sci.* 69, 223–241. doi: 10.1071/CP17387
- Molla, K. A., and Yang, Y. (2019). CRISPR/Cas-mediated base editing: Technical considerations and practical applications. *Trends Biotechnol.* 37, 1121–1142. doi: 10.1016/j.tibtech.2019.03.008
- Mondal, S., Dutta, S., Crespo-Herrera, L., Huerta-Espino, J., Braun, H. J., and Singh, R. P. (2020). Fifty years of semi-dwarf spring wheat breeding at CIMMYT: Grain yield progress in optimum, drought and heat stress environments. *F. Crop Res.* 250, 107757. doi: 10.1016/j.fcr.2020.107757
- Monneveux, P., Reynolds, M. P., Zaharieva, M., and Mujeeb-Kazi, A. (2003). Effect of T1BL1RS chromosome translocation on bread wheat grain yield and physiological related traits in a warm environment. *Cereal Res. Commun.* 31, 371–378. doi: 10.1007/bf03543367
- Mwadingeni, L., Shimelis, H., Dube, E., Laing, M. D., and Tsilo, T. J. (2016a). Breeding wheat for drought tolerance: Progress and technologies. *J. Integr. Agric.* 15, 935–943. doi: 10.1016/S2095-3119(15)61102-9
- Mwadingeni, L., Shimelis, H., Tesfay, S., and Tsilo, T. J. (2016b). Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Front. Plant Sci.* 7, 1276. doi: 10.3389/fpls.2016.01276
- Mwadingeni, L., Shimelis, H., Rees, D. J. G., and Tsilo, T. J. (2017). Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. *PLoS One* 12, e0171692. doi: 10.1371/journal.pone.0171692
- Nagy, É., Lehoczki-Krsjak, S., Lantos, C., and Pauk, J. (2018). Phenotyping for breeding drought tolerance on wheat varieties of different origins. *South Afr. J. Bot.* 116, 216–221. doi: 10.1016/j.sajb.2018.03.009
- Nemtsev, B. F., Nemtsev, A. B., Goncharov, N. P., and Kurkova, S. V. (2019). Spring common wheat breeding lines produced on the basis of distant hybridization: ecological strain testing in Bagan. *Curr. Challenges Plant Genet. Genom. Bioinform. Biotechnol.* 2019, 30–33. doi: 10.18699/icg-plantgen2019-07
- Nezhad, K. Z., Weber, W. E., Röder, M. S., Sharma, S., Lohwasser, U., Meyer, R. C., et al. (2012). QTL analysis for thousand-grain weight under terminal drought stress in bread wheat (*Triticum aestivum* L.). *Euphytica* 186, 127–138. doi: 10.1007/s10681-011-0559-y
- Niemirowicz-Szczytt, K. (1997). Excessive homozygosity in doubled haploids - Advantages and disadvantages for plant breeding and fundamental research. *Acta Physiol. Plant* 19, 155–167. doi: 10.1007/s11738-997-0032-7
- Okada, A., Arndell, T., Borisjuk, N., Sharma, N., Watson-haigh, N. S., Tucker, E. J., et al. (2019). CRISPR/Cas9-mediated knockout of Ms1 enables the rapid generation of male-sterile hexaploid wheat lines for use in hybrid seed production. *Plant Biotechnol. J.* 17, 1905–1913. doi: 10.1111/pbi.13106
- Padulosi, S., Bergamini, N., and Lawrence, T. (2012). “On-farm conservation of neglected and underutilized species: Status, trends, and novel approaches to deal with climate change.” *Proceedings of the International Conference Friedrichsdorf, Frankfurt*, 14–16 June, 2011 (Bioversity International, Rome), 307.
- Petrov, P., Petrova, A., Tashev, I. D. T., Brestic, K. O. M., and Misheva, S. (2018). Relationships between leaf morpho-anatomy, water status and cell membrane stability in leaves of wheat seedlings subjected to severe soil drought. *J. Agron. Crop Sci.* 204, 219–227. doi: 10.1111/jac.12255
- Pinto, R. S., Reynolds, M. P., Mathews, K. L., McIntyre, C. L., Olivares-Villegas, J. J., and Chapman, S. C. (2010). Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. *Theor. Appl. Genet.* 121, 1001–1021. doi: 10.1007/s00122-010-1351-4
- Poland, J., Endelman, J., Dawson, J., Rutkoski, J., Wu, S. Y., Manes, Y., et al. (2012). Genomic selection in wheat breeding using genotyping-by-sequencing. *Plant Genome* 5, 103–113. doi: 10.3835/Plantgenome2012.06.0006
- Qaseem, M. F., Qureshi, R., and Muqaddasi, Q. H. (2018). Genome-wide association mapping in bread wheat subjected to independent and combined high temperature and drought stress. *PLoS One* 13, e0199121. doi: 10.1371/journal.pone.0199121
- Qaseem, M. F., Qureshi, R., Shaheen, H., and Shafiq, N. (2019). Genome-wide association analyses for yield and yield-related traits in bread wheat (*Triticum aestivum* L.) under pre-anthesis combined heat and drought stress in field conditions. *PLoS One* 14, e0213407. doi: 10.1371/journal.pone.0213407
- Raina, A., Laskar, R., Khursheed, S., Amin, R., Tantray, Y., Parveen, K., et al. (2016). Role of mutation breeding in crop improvement- past, present and future. *Asian Res. J. Agric.* 2, 1–13. doi: 10.9734/arja/2016/29334
- Rajaram, S. (2005). Role of conventional plant breeding and biotechnology in future wheat production. *Turkish J. Agric. For.* 29, 105–111. doi: 10.3906/tar-0404-1
- Ramirez-Gonzalez, R. H., Segovia, V., Bird, N., Caccamo, M., and Uauy, C. (2015). “Next generation sequencing enabled genetics in hexaploid wheat,” in *Advances in Wheat Genetics: From Genome to Field. Proceedings of 12th International Wheat Genetics Symposium*. Eds. Y. Ogihara, S. Takumi and H. Handa (Yokohama, Japan: Springer Open), 201–209. Available at: <https://www.springer.com/gp/book/9784431556749>.
- Ramya, P., Singh, G. P., Jain, N., Singh, P. K., Pandey, M. K., Sharma, K., et al. (2016). Effect of recurrent selection on drought tolerance and related morpho-physiological traits in bread wheat. *PLoS One* 11, e0156869. doi: 10.1371/journal.pone.0156869

- Rauf, S., Teixeira, J. A., Ali, A., and Abdul, K. (2010). Consequences of plant breeding on genetic diversity. *Int. J. Plant Breed.* 4, 1–21.
- Ray, S., and Satya, P. (2014). Next generation sequencing technologies for next generation plant breeding. *Front. Plant Sci.* 5, 367. doi: 10.3389/fpls.2014.00367
- Rebetzke, G. J., Richards, R. A., Fischer, V. M., and Mickelson, B. J. (1999). Breeding long coleoptile, reduced height wheats. *Euphytica* 106, 159–168. doi: 10.1023/A:1003518920119
- Rebetzke, G. J., Appels, R., Morrison, A. D., Richards, R. A., McDonald, G., Ellis, M. H., et al. (2001). Quantitative trait loci on chromosome 4B for coleoptile length and early vigour in wheat (*Triticum aestivum* L.). *Aust. J. Agric. Res.* 52, 1221–1234. doi: 10.1071/ar01042
- Rebetzke, G. J., Ellis, M. H., Bonnett, D. G., and Richards, R. A. (2007). Molecular mapping of genes for Coleoptile growth in bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 114, 1173–1183. doi: 10.1007/s00122-007-0509-1
- Rebetzke, G. J., Condon, A. G., Farquhar, G. D., Appels, R., and Richards, R. A. (2008). Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. *Theor. Appl. Genet.* 118, 123–137. doi: 10.1007/s00122-008-0882-4
- Reddy, S. K., Liu, S., Rudd, J. C., Xue, Q., Payton, P., Finlayson, S. A., et al. (2014). Physiology and transcriptomics of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. *J. Plant Physiol.* 171, 1289–1298. doi: 10.1016/j.jplph.2014.05.005
- Ren, T., Tang, Z., Fu, S., Yan, B., Tan, F., Ren, Z., et al. (2017). Molecular cytogenetic characterization of novel wheat-rye T1RS.1BL translocation lines with high resistance to diseases and great agronomic traits. *Front. Plant Sci.* 8, 799. doi: 10.3389/fpls.2017.00799
- Reynolds, M., Dreccer, F., and Trethowan, R. (2007). Drought-adaptive traits derived from wheat wild relatives and landraces. *J. Exp. Bot.* 58, 177–186. doi: 10.1093/jxb/erl250
- Rosyara, U., Kishii, M., Payne, T., Sansaloni, C. P., Singh, R. P., Braun, H. J., et al. (2019). Genetic contribution of synthetic hexaploid wheat to CIMMYT's spring bread wheat breeding germplasm. *Sci. Rep.* 9, 12355. doi: 10.1038/s41598-019-47936-5
- Saeidi, M., and Abdoli, M. (2015). Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. *J. Agric. Sci. Technol.* 17, 885–898.
- Saeidi, M., Ardalani, S., Jalali-Honarmand, S., Ghobadi, M. E., and Abdoli, M. (2015). Evaluation of drought stress at vegetative growth stage on the grain yield formation and some physiological traits as well as fluorescence parameters of different bread wheat cultivars. *Acta Biol. Szeged* 59, 35–44.
- Sallam, A., Arbaoui, M., El-Esawi, M., Abshire, N., and Martsch, R. (2016). Identification and verification of QTL associated with frost tolerance using linkage mapping and GWAS in winter Faba bean. *Front. Plant Sci.* 7, 1098. doi: 10.3389/fpls.2016.01098
- Sareen, S., Sharma, P., Tiwari, V., and Sharma, I. (2014). Identifying wheat landraces as genetic resources for drought and heat tolerance. *Res. Crop* 15, 846–851. doi: 10.5958/2348-7542.2014.01421.1
- Sarto, M. V. M., Sarto, J. R. W., Rampim, L., Bassegio, D., da Costa, P. F., and Inagaki, A. M. (2017). Wheat phenology and yield under drought: A review. *Aust. J. Crop Sci.* 11, 941–946. doi: 10.21475/ajcs17.11.08.pne351
- Scherer, A., and Christensen, G. B. (2016). Concepts and relevance of genome-wide association studies. *Sci. Prog.* 99, 59–67. doi: 10.3184/003685016X14558068452913
- Schmidt, J., Tricker, P. J., Eckermann, P., Kalambettu, P., Garcia, M., and Fleury, D. (2020). Novel alleles for combined drought and heat stress tolerance in wheat. *Front. Plant Sci.* 10, 1800. doi: 10.3389/fpls.2019.01800
- Sehgal, D., Vikram, P., Sansaloni, C. P., Ortiz, C., Pierre, C., Saint, et al. (2015). Exploring and mobilizing the gene bank biodiversity for wheat improvement. *PLoS One* 10, e0132112. doi: 10.1371/journal.pone.0132112
- Sen, A., Ozturk, I., Yaycili, O., and Alikamanoglu, S. (2017). Drought tolerance in irradiated wheat mutants studied by genetic and biochemical markers. *J. Plant Growth Regul.* 36, 669–679. doi: 10.1007/s00344-017-9668-8
- Shamasbi, F. V., Jamali, S. H., Sadeghzadeh, B., and Mandoulakani, B. A. (2017). Genetic mapping of quantitative trait loci for yield-affecting traits in a barley doubled haploid population derived from clipper × sahara 3771. *Front. Plant Sci.* 8, 688. doi: 10.3389/fpls.2017.00688
- Shi, X., and Ling, H. (2017). Current advances in genome sequencing of common wheat and its ancestral species. *Crop J.* 6, 15–21. doi: 10.1016/j.cj.2017.11.001
- Shi, Y., Thomasson, J. A., Murray, S. C., Pugh, N. A., Rooney, W. L., Shafian, S., et al. (2016). Unmanned aerial vehicles for high-throughput phenotyping and agronomic research. *PLoS One* 11, e0159781. doi: 10.5061/dryad.65m87
- Shi, W., Hao, C., Zhang, Y., Cheng, J., Zhang, Z., Liu, J., et al. (2017). A combined association mapping and linkage analysis of kernel number per spike in common wheat (*Triticum aestivum* L.). *Front. Plant Sci.* 8, 1412. doi: 10.3389/fpls.2017.01412
- Shi, S., Azam, F. II, Li, H., Chang, X., Li, B., and Jing, R. (2017). Mapping QTL for stay-green and agronomic traits in wheat under diverse water regimes. *Euphytica* 213, 246. doi: 10.1007/s10681-017-2002-5
- Shinwari, Z. K., Jan, S. A., Nakashima, K., and Yamaguchi-Shinozaki, K. (2020). Genetic engineering approaches to understanding drought tolerance in plants. *Plant Biotechnol. Rep.* 14, 151–162. doi: 10.1007/s11816-020-00598-6
- Shrestha, R., Matteis, L., Skofic, M., Portugal, A., McLaren, G., Hyman, G., et al. (2012). Bridging the phenotypic and genetic data useful for integrated breeding through a data annotation using the Crop Ontology developed by the crop communities of practice. *Front. Physiol.* 3, 326. doi: 10.3389/fphys.2012.00326
- Singh, R. P., Huerta-Espino, J., Rajaram, S., and Crossa, J. (1998). Agronomic effects from chromosome translocations 7DL.7AG and 1BL.1RS in spring wheat. *Crop Sci.* 38, 27–33. doi: 10.2135/cropsci1998.0011183X003800010005x
- Singh, A., Ganapathysubramanian, B., Singh, A. K., and Sarkar, S. (2016). Machine learning for high-throughput stress phenotyping in plants. *Trends Plant Sci.* 21, 110–124. doi: 10.1016/j.tplants.2015.10.015
- Singh, B., Kukreja, S., and Goutam, U. (2018). Milestones achieved in response to drought stress through reverse genetic approaches. *F1000Research* 7, 1311. doi: 10.12688/f1000research.15606.1
- Singh, S., Vikram, P., Sehgal, D., Burgueño, J., Sharma, A., Singh, S. K., et al. (2018). Harnessing genetic potential of wheat germplasm banks through impact-oriented-prebreeding for future food and nutritional security. *Sci. Rep.* 8, 12527. doi: 10.1038/s41598-018-30667-4
- Singh, N., Wu, S., Raupp, W. J., Sehgal, S., Arora, S., Tiwari, V., et al. (2019). Efficient curation of genebanks using next generation sequencing reveals substantial duplication of germplasm accessions. *Sci. Rep.* 9, 650. doi: 10.1038/s41598-018-37269-0
- Sohail, Q., Inoue, T., Tanaka, H., Eltayeb, A. E., Matsuoka, Y., and Tsujimoto, H. (2011). Applicability of *Aegilops tauschii* drought tolerance traits to breeding of hexaploid wheat. *Breed. Sci.* 61, 347–357. doi: 10.1270/jsbbs.61.347
- Song, Q., Liu, C., Goudia, D., Chen, L., and Hu, Y. (2017). Drought resistance of new synthetic hexaploid wheat accessions evaluated by multiple traits and antioxidant enzyme activity. *F. Crop Res.* 210, 91–103. doi: 10.1016/j.fcr.2017.05.028
- Soriano, J. M., and Alvaro, F. (2019). Discovering consensus genomic regions in wheat for root-related traits by QTL meta-analysis. *Sci. Rep.* 9, 10537. doi: 10.1038/s41598-019-47038-2
- Spilmeyer, W., Hyles, J., Joaquim, P., Azanza, F., Bonnett, D., Ellis, M. E., et al. (2007). A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. *Theor. Appl. Genet.* 115, 59–66. doi: 10.1007/s00122-007-0540-2
- Su, Y., Wu, F., Ao, Z., Jin, S., Qin, F., Liu, B., et al. (2019). Evaluating maize phenotype dynamics under drought stress using terrestrial lidar. *Plant Methods* 15, 11. doi: 10.1186/s13007-019-0396-x
- Suneja, Y., Gupta, A. K., and Bains, N. S. (2019). Stress adaptive plasticity: *Aegilops tauschii* and *Triticum dicoccoides* as potential donors of drought associated morpho-physiological traits in wheat. *Front. Plant Sci.* 10, 211. doi: 10.3389/fpls.2019.00211
- Szabo-Hever, A., Zhang, Q., Friesen, T. L., Zhong, S., Elias, E. M., Cai, X., et al. (2018). Genetic diversity and resistance to Fusarium head blight in synthetic hexaploid wheat derived from *Aegilops tauschii* and diverse *Triticum turgidum* subspecies. *Front. Plant Sci.* 871, 1829. doi: 10.3389/fpls.2018.01829
- Tahmasebi, S., Heidari, B., Pakniyat, H., and Dadkhodaie, A. (2015). Consequences of 1BL/1RS translocation on agronomic and physiological traits in wheat. *Cereal Res. Commun.* 43, 554–566. doi: 10.1556/0806.43.2015.016
- Touzy, G., Rincint, R., Bogard, M., Lafarge, S., Dubreuil, P., Mini, A., et al. (2019). Using environmental clustering to identify specific drought tolerance QTLs in bread wheat (*T. aestivum* L.). *Theor. Appl. Genet.* 132, 2859–2880. doi: 10.1007/s00122-019-03393-2

- Tura, H., Edwards, J., Gahlaut, V., Garcia, M., Sznajder, B., Baumann, U., et al. (2020). QTL analysis and fine mapping of a QTL for yield-related traits in wheat grown in dry and hot environments. *Theor. Appl. Genet.* 133, 239–257. doi: 10.1007/s00122-019-03454-6
- Uauy, C. (2017). Wheat genomics comes of age. *Curr. Opin. Plant Biol.* 36, 142–148. doi: 10.1016/j.pbi.2017.01.007
- United Nations (2019). *World Population Prospects 2019: Highlights (ST/ESA/SER.A/423)* (United Nations: Department of Economic and Social Affairs, Population Division). Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12283219>.
- Verma, V., Foulkes, M. J., Sylvester-Bradley, R., Caligari, P. D. S., and Snape, J. W. (2004). Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought stressed environments. *Euphytica* 135, 255–263.
- Vikram, P., Franco, J., Burgueño-Ferreira, J., Li, H., Sehgal, D., Saint Pierre, C., et al. (2016). Unlocking the genetic diversity of Creole wheats. *Sci. Rep.* 6, 23092. doi: 10.1038/srep23092
- Visscher, P. M., Wray, N. R., Zhang, Q., Sklar, P., McCarthy, M. II, Brown, M. A., et al. (2017). 10 years of GWAS discovery: biology, function, and translation. *Am. J. Hum. Genet.* 101, 5–22. doi: 10.1016/j.ajhg.2017.06.005
- Wang, M., Jiang, N., Jia, T., Leach, L., Cockram, J., Waugh, R., et al. (2012). Genome-wide association mapping of agronomic and morphologic traits in highly structured populations of barley cultivars. *Theor. Appl. Genet.* 124, 233–246. doi: 10.1007/s00122-011-1697-2
- Wang, X., Vignjevic, M., Liu, F., Jacobsen, S., Jiang, D., and Wollenweber, B. (2015). Drought priming at vegetative growth stages improves tolerance to drought and heat stresses occurring during grain filling in spring wheat. *Plant Growth Regul.* 75, 677–687. doi: 10.1007/s10725-014-9969-x
- Wang, M., Wang, S., Liang, Z., Shi, W., Gao, C., and Xia, G. (2018). From genetic stock to genome editing: gene exploitation in wheat. *Trends Biotechnol.* 36, 160–172. doi: 10.1016/j.tibtech.2017.10.002
- White, J. W., Andrade-Sanchez, P., Gore, M. A., Bronson, K. F., Coffelt, T. A., Conley, M. M., et al. (2012). Field-based phenomics for plant genetics research. *Field Crop Res.* 133, 101–112. doi: 10.1016/j.fcr.2012.04.003
- Yan, G., Liu, H., Wang, H., Lu, Z., Wang, Y., Mullan, D., et al. (2017). Accelerated generation of selfed pure line plants for gene identification and crop breeding. *Front. Plant Sci.* 8, 1786. doi: 10.3389/fpls.2017.01786
- Yang, S., Vanderbeld, B., Wan, J., and Huang, Y. (2010). Narrowing down the targets: Towards successful genetic engineering of drought-tolerant crops. *Mol. Plant* 3, 469–490. doi: 10.1093/mp/ssp016
- Yang, W., Feng, H., Zhang, X., Zhang, J., Doonan, J. H., Batchelor, W. D., et al. (2020). Crop phenomics and high-throughput phenotyping: Past decades, current challenges, and future perspectives. *Mol. Plant* 13, 187–214. doi: 10.1016/j.molp.2020.01.008
- Yu, T. F., Xu, Z. S., Guo, J. K., Wang, Y. X., Abernathy, B., Fu, J. D., et al. (2017). Improved drought tolerance in wheat plants overexpressing a synthetic bacterial cold shock protein gene *SeCspA*. *Sci. Rep.* 7, 44050. doi: 10.1038/srep44050
- Zandipour, M., Majidi Hervan, E., Azadi, A., Khosroshahli, M., and Etminan, A. (2020). A QTL hot spot region on chromosome 1B for nine important traits under terminal drought stress conditions in wheat. *Cereal Res. Commun.* 48, 17–24. doi: 10.1007/s42976-020-00017-0
- Zhang, J., and Kirkham, M. B. (1994). Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol.* 35, 785–791. doi: 10.1093/oxfordjournals.pcp.a078658
- Zhang, H., Cui, F., Wang, L., Li, J., Ding, A., Zhao, C., et al. (2013). Conditional and unconditional QTL mapping of drought-tolerance-related traits of wheat seedling using two related RIL populations. *J. Genet.* 92, 213–231. doi: 10.1007/s12041-013-0253-z
- Zhang, J., Xu, Y., Chen, W., Dell, B., Vergauwen, R., Biddulph, B., et al. (2015). A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought. *New Phytol.* 205, 293–305. doi: 10.1111/nph.13030
- Zhang, X., Huang, C., Wu, D., Qiao, F., Li, W., Duan, L., et al. (2017). High-throughput phenotyping and QTL mapping reveals the genetic architecture of maize. *Plant Physiol.* 173, 1554–1564. doi: 10.1104/pp.16.01516
- Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., et al. (2017). Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *Plant J.* 91, 714–724. doi: 10.1111/tpj.13599
- Zhang, Y., Li, D., Zhang, D., Zhao, X., Cao, X., Dong, L., et al. (2018). Analysis of the functions of TaGW2 homoeologs in wheat grain weight and protein content traits. *Plant J.* 94, 857–866. doi: 10.1111/tpj.13903
- Zhang, Y., Wang, Z., Fan, Z., Li, J., Gao, X., Zhang, H., et al. (2019). Phenotyping and evaluation of CIMMYT WPHYSGP nursery lines and local wheat varieties under two irrigation regimes. *Breed. Sci.* 67, 55–67. doi: 10.1270/jsbbs.18104
- Zhao, X., Luo, L., Cao, Y., Liu, Y., Li, Y., Wu, W., et al. (2018). Genome-wide association analysis and QTL mapping reveal the genetic control of cadmium accumulation in maize leaf. *BMC Genomics* 19, 91. doi: 10.1186/s12864-017-4395-x
- Zong, Y., Wang, Y., Li, C., Zhang, R., Chen, K., Ran, Y., et al. (2017). Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat. Biotechnol.* 35, 438–440. doi: 10.1038/nbt.3811

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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