Genetic Improvement of Wheat for Drought Tolerance

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Wheat production and productivity are challenged by recurrent droughts associated with climate change globally. Drought and heat stress resilient cultivars can alleviate yield loss in marginal production agro-ecologies. The ability of some crop genotypes to thrive and yield in drought conditions is attributable to the inherent genetic variation and environmental adaptation, presenting opportunities to develop drought-tolerant varieties. Understanding the underlying genetic, physiological, biochemical, and environmental mechanisms and their interactions is key critical opportunity for drought tolerance improvement.

1. Introduction

Wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is a key commodity crop driving food security and the global economy along the value chains. With an increasing world human population and dwindling agricultural lands, the global demand for wheat products will increase by 60% by 2050 [1]. Therefore, wheat yields will need to increase by 1.6% per annum to meet world demands. Recurrent droughts attributable to climate change affect agriculture-based systems through unpredictable rainfall patterns and changes in crop cycles, diseases, and pest dynamics, leading to a reduction in potential yield gains. Consequently, this compromises food security and economic development, among others [2][3].

Low productivity presents a great challenge to wheat farmers due to market fluctuations and price shocks [4]. Hence, there is a need to develop and adopt drought-adapted modern cultivars that are climate-resilient to mitigate the impacts of current and future environmental changes and deliver market-preferred products. Creating drought-tolerant, high yielding and water-use efficient cultivars is the most economical efficient strategy. The use of irrigation water is unaffordable and unsustainable, especially in arid and semi-arid regions, including sub-Saharan African countries. Elucidation of the genetic, agronomic, and environmental components is paramount to determining the response of wheat to drought. Furthermore, the exploitation of genetic resources such as landraces, synthetics and wild relatives will lead to the discovery of a wealth of essential alleles for wheat improvement programs.

Drought stress hampers plant growth, development and yield by changing the inherent agro-physiological and biochemical processes and pathways [5][6]. Drought stress occurs in different patterns and intensities at different crop growth stages [2][7][8]. The impact of drought stress differs according to the genotype, environment and genotype x environment interaction [9]. In addition, drought tolerance is a complex quantitative trait governed by multiple agronomic traits and polygenes [10]. This has posed significant limitations in developing breeding populations and varieties with improved drought tolerance. The temporal and spatial variation in imposing drought stress across different environments and experiments has contributed to poor selection efficiency, slowing genetic progress in drought tolerance breeding. The essential target agronomic traits for improving drought tolerance include early heading, anthesis and maturity [11][12][13][14], as well as root system architecture [15][16]. Physiological traits include relative water content (RWC), canopy temperature (CT), normalized difference vegetative index (NDVI), stem water-soluble carbohydrates (WSC), among others [5][6][17][18][19]. Further, the main biochemical traits associated with drought tolerance include high soluble sugar content, chlorophyll content, reduced gas exchange, high proline content, increased carbohydrates content and reduced superoxide dismutase concentration [6][20][21]. Ideotype breeding with desirable agro-morphological, physiological and biochemical traits can potentially improve drought tolerance in wheat.

2. Impact of Drought Stress on Wheat Production

Drought stress has a catastrophic effect on agricultural production. Agronomic traits such as grain yield were reportedly reduced by 25% to 62.75%, grain numbers per spike by 38% to 50%, 1000-kernel weight by 16.4% to 19.42% and plant height by 14.7% to 34.45% under drought stress across different studies [5][6][22][23][24]. This suggests that grain yield and grain numbers per spike are more sensitive to drought stress. Physiological traits such as leaf water content,

photosynthetic rate and chlorophyll content reduced by up to 73.8%, 32% and 19%, respectively, suggesting that leaf water content was more sensitive to drought stress [25][26]. Root biomass and above-ground plant biomass were reduced by 23% and 45%, in that order [27][28]. Hence, there is a need to breed for new varieties that can maintain or partition more biomass under drought stress. A meta-analysis from 144 studies published between 1980 and 2015 showed that wheat yields have declined by 20.6% [2], while a related study that included 60 published studies between 1980 and 2017 showed a reduction of 27.5% due to drought stress at different growth stages [23]. Recently, several countries in Africa experienced drought events which reduced wheat production by 45% [29]. Several factors such as increased population growth, unsustainable agricultural production and ecological imbalance (soil erosion, depletion of nutrients and water resources, overutilization of natural vegetation, and environmental disasters) have aggravated the impact of drought stress in these countries [30].

Drought stress affects wheat at all stages of crop growth. However, its effect is more devastating at the seedling, tillering, jointing, heading, anthesis and grain filling stages [4][23][31]. Drought at the seedling stage can inflict up to a 50% reduction in root length [32]. Notably, reproductive and grain filling stages are the most sensitive stages to water stress [33][34][35][36]. Drought at anthesis causes abortion of ovules, consequently reducing the number of grains per spike and grain weight and ultimately grain yield [5][37][38]. During grain filling stage, moisture deficit may disrupt nutrient uptake and photosynthesis, leading to the production of shrivelled kernels [35]. Bennett et al. [39] reported that drought stress reduced yield by 65%. The authors reported a reduction in the heritability values of grain yield from 74% to 58% due to drought. Plants show a reduced stomatal opening and gaseous exchange when stressed, which leads to low photosynthetic efficiency and yield gains. Thus, drought stress will inhibit trait inheritance, photosynthesis and grain yield.

3. Challenges in Breeding for Drought Tolerance

Recurrent drought has become the most prominent cause of reduced yield, grain quality and threat to food security and livelihoods [40]. Challenges in breeding for drought tolerance were reported. There are limited research efforts that identified key root traits in selecting and improving drought-tolerant wheat. Notably, improved root system attributes (e.g., deep and wide-spreading) are desirable for breeding drought-tolerant wheat cultivars [41]. Nevertheless, there is a need to identify key root traits to improve or develop cultivars with improved root attributes and aid in marker-assisted selection. Complementarily, there is a lack of simple and efficient phenotyping methods to improve root attributes as they are labor-intensive and require destructive sampling [42][43]. Developing new high-throughput phenotyping methods that will promote systematic phenotyping of root attributes is of paramount importance. There are limited research efforts that identified key agro-physiological traits in selecting and improving drought-tolerant crops. This is because most drought-adaptive and constitutive traits are controlled by polygenic epistatic and unstable quantitative trait loci (QTL), which are highly influenced by genotype-environment interaction [10][20][44][45][46][47]. This renders low selection efficiency for superior genotypes [48]. Genotype–environment interactions are manifested through crossover ranking and rank inconsistencies when using different indices in identifying drought-tolerant genotypes [49]. Identifying genes associated with drought stress tolerance and their expression and bridging the gap between theoretical research and applied crop breeding is another challenge for brought tolerance breeding [50]. This can be tackled by establishing concerted research groups to reveal the genetic, epigenetic, transcriptomic and metabolomic bases of agro-physiological and root attributes associated with drought tolerance in wheat [51][52][53]. The large genome size (17 Gb) of wheat makes it comparatively more difficult to identify genetic loci controlling key agro-physiological traits conferring drought tolerance in wheat due to its complex genetic background. In this regard, identifying stable QTL or establishing marker–trait associations under contrasting water regimes is crucial for improving drought tolerance using marker-assisted selection (MAS). Extending the genetic analysis research into applied breeding beyond QTL detection has been minimal due to the lack of robust phenotyping and the need for translational genetics. Furthermore, existing mapping populations are routinely used, needing the development of new drought-suited populations. Therefore, new mapping populations sourced from genetically and complementary genotypes will provide avenues for improved drought tolerance [54].

4. Opportunities for Drought Tolerance Breeding

4.1. Mechanisms of Drought Tolerance

There are different mechanisms of drought response, including drought escape, drought avoidance and drought tolerance [12][33]. Drought escape is an adaptive trait that enables the plants to grow and complete their life cycle before the beginning of severe drought [55]. Early heading, flowering and maturity, reduced plant height and short growth cycle are unique attributes to escape dry spells [11][12][13]. In particular, early heading, flowering and maturity are major drought escape mechanisms that allow the completion of the life cycle before the onset of terminal drought stress, which is common in most rainfed agro-systems [14]. Drought avoidance includes the ability for enhanced uptake of available water

and nutrients by a longer or deeper root system [13]. This mechanism is associated with a slow growth rate, small or closed stomata, decreased leaf area, reduced photosynthetic activity and low cell metabolism [55]. Root traits such as increased root biomass, root length density and rooting depth are key drivers of drought avoidance [56]. Decreased leaf area is one of the drought avoidance attributes which results in reduced water loss through transpiration [57]. Drought tolerance is the ability of the plant to maintain its growth, development and reproduction under drought stress conditions [12]. Early maturity and reduced leaf area are common attributes in drought-adapted genotypes. Therefore, understanding plant response to drought tolerance at all growth stages is paramount for breeding.

4.2 Selection Indices for Drought Tolerance

Use of the target selection and production environments and water stress management remain fundamental approaches in drought tolerance improvement [30][58]. Abdolshahi et al. [42] reported three approaches for breeding drought tolerance. These include (1) breeding for higher yield under non-stress conditions, (2) breeding for maximum yield under drought-prone environments and (3) breeding for drought tolerance using selection indices (traits). For enhanced selection efficiency under non-stress conditions aimed at improving performance under the target drought-prone environment, the procedure assumes the trait(s) measured in two different environments not as one but as two traits correlated genetically. This is because the physiological and genetic mechanisms and the genes required for superior performance may be different under these environments [30]. High genetic correlation of traits under complementary selection environments guarantees higher selection responses for yield and yield influencing traits. Traits with high heritability, genetic advance and genetic gains are essential for direct and indirect selection for better grain yield under different environmental conditions [59][60][61]. Indirect selection involves a selection of one trait via another, while direct selection involves the per se selection of the target trait [30]. The use of integrative traits accompanied by the development and application of new and advanced technologies could accelerate the phenotypic selection of drought adaptive traits and consequently improve yield in marginal/low-yielding environments.

Agronomic traits such as early heading, anthesis, maturity, spike morphology and reduced plant height have been widely targeted in drought tolerance breeding programs [21][36][62][63]. These traits have been used in direct or indirect selection for grain yield and drought tolerance in wheat [21][24][36][49][60]. Drought response varies across the source populations. Hence evaluating each population is necessary for simultaneous improvement of yield and drought tolerance.

Some physiological traits have been recognised as reliable, cost-effective and non-invasive methods for automated high-throughput phenotyping in crop breeding programs. According to Monneveux et al. [58], physiological traits can be used to select parental genotypes to be used in cross formation. Hence, physiological traits are useful as direct selection criteria for screening populations to eliminate undesirable segregants across generations. Sallam et al. [64] have extensively reviewed drought tolerance-related physiological traits and advances in breeding and genetics research. Key physiological traits are osmotic potential, stay-green, leaf area, relative water content, canopy temperature, normalised difference vegetative index (NDVI), leaf water status and stem water-soluble carbohydrates (WSC) [5][6][17][18]. Stay-green is the ability of a genotype to remain green and continue undertaking photosynthesis due to higher chlorophyll content compared with other genotypes under drought stress [65]. Such genotypes have improved performance under drought conditions with higher grain yield and biomass production [66][67]. SeriM82, a high yielding cultivar released in 1982 exhibited a stay-green phenotype by maintaining green leaf area longer during the grain filling [68]. NDVI is an indirect selection method for stay-green and yield potential [69], while CT denotes the plant's interaction with the soil and atmosphere whereby plants can mine water under water-restricted conditions [70]. Osmotic adjustments occur when molecular weight accumulates in lower levels of organic solutes [71]. Leaf water status depends on the cell osmotic conditions and water transportation from plant shoots [72]. Canopy temperature [6] and RWC [36] were major yield determinants. Grain yield was associated with CT at both vegetative and grain filling stages [46] and NDVI under rainfed conditions [19]. Furthermore, stem WSC remobilisation during grain filling contributes to grain yield under drought stress [73].

Relatively better yield under drought stress can be achieved by incorporating drought-adaptive biochemical traits from genetically diverse and unrelated parents [24]. Some of the biochemical traits for drought tolerance include soluble sugar content, chlorophyll content, gas exchange, proline content, carbohydrate content, and superoxide dismutase concentration [6][20][21]. Proline content regulates nitrogen accumulation and contributes to membrane stability [74]. Gas exchange is among the key traits susceptible to drought stress. Gas exchange parameters include photosynthetic rate, stomatal conductance, chlorophyll content and water use efficiency [75]. Drought stress tolerance in wheat was associated with high antioxidant enzyme activity, i.e., catalase, glutathione reductase and peroxidase and elevated S-metabolites, i.e., methionine cysteine and glutathione [76][77]. Synthetic derivatives (SYN-DERs) accumulated more soluble sugars, superoxide dismutase concentration, and proline content under drought stress [6]. Proline content was significantly

correlated with grain yield suggesting selection efficiency of this trait under drought stress [20]. A reduction in chlorophyll content denotes decreased photosynthesis efficiency [78].

The root and shoot systems are vital for plant growth and development. Exploring genotype plasticity for roots and shoots is useful to improve drought tolerance [28][60][78]. The shoot systems influence plant adaptive response due to differential environmental changes, including drought stress [41]. Roots are primary organs necessary for growth resource acquisition such as water and minerals; thus, wheat varieties with broad environmental adaption and high water use efficiency are the best candidates for breeding. As the soil dries at the surface, a wider and deeper root system ensures access to soil moisture deeper in the profile during water deficit [79]. Thus, breeding for traits such as root length, density, volume, surface area and diameter is an efficient strategy in environments where water deeper in the profile could be available later in the growing season [80][81]. These traits are indirectly involved in water and nutrient acquisition for plant growth [82]. Root length and surface area denote the ability of the plant to acquire soil resources (water and nutrients) [82]. The root length density is used to estimate the soil volume explored by the plant root architecture and consequently the amount of resources (water and nutrients) available to the plant in the soil [80][83]. Root diameter reflects the ability of the plant to adapt to changes in temperature [89], soil texture and water content [84], and mycorrhizal status [84]. This highlights the importance of identifying root system attributes that provide better exploration of the soil profile for resource acquisition and storage, and plant anchorage.

Compared with shallow root genotypes, deep-rooted types have larger-sized grains, higher grain weight and yield. El-Hassouni et al. [85] reported that thousand-kernel weight was 9% higher while grain size and grain yield were 35% higher in deeper rooted genotypes. Root traits can influence stay-green attributes and adaptation to a wide range of climatic or soil conditions [65][86]. Thus, phenotyping of both stay-green and root traits could enable the selection of superior phenotypes for either broad or specific water-stress adaptation.

Plant response or adaptation to drought partially depends on soil properties and the soil water status. Some genotypes respond to drought by maintaining or increasing root growth while decreasing shoot growth [27][87]. Reduced root growth may also occur due to low water status, low oxygen levels (hypoxia or anoxia) and high soil impedance [88]. Early sowing ensures more profound root growth with the ability to access water from deeper soil profiles in drought-prone environments [89]. In this case, increased root versus shoot growth may improve the plant water status under different drought patterns due to enhanced acquisition of water to produce more root tips and maintain the existing shoots. *GmbZIP1* has been linked to increased root and shoot growth under drought [90]. Above-ground biomass was reduced by 45% under drought stress compared to non-stressed conditions in Akron, Ohio, USA [27]. Fletcher and Chenu [91][92] investigated the biomass partitioning of 15 elite Australian cultivars released between 1973 and 2012 and found non-significant changes in the plant biomass and green-leaf biomass at flowering. Only new varieties partitioned more stem and spike biomass at the expense of reduced leaves. Changes in tillering ability have also played a role in the partitioning of stems and spikes. For example, during the 1970s, modern varieties showed increased biomass for every litre of water transpired due to high tillering [92], saving up to 500 g of water per plant up to flowering. According to Kirkegaard et al. [93], a 30 cm increase in root depth into the subsoil could extract an extra 10 mm of water in the deeper soils. *VRN1* is a gene modulating flowering behavior and balance between shoot and root architecture in wheat and barley [94][95]. A spring wheat cultivar Dharwar Dry released in 1994 exhibited superior shoot and root attributes when assessed at different growth stages under multiple growth conditions. This variety can improve root traits in dryland or water-stressed conditions [96]. There have been fewer studies on genetic analysis of root and shoot attributes due to difficulties in phenotyping as they are labour-intensive and require destructive sampling [42][43]. Genetic variation for phenotypic plasticity is threatened by directional selection within a narrow gene pool composed mainly of elite lines. Yet it is imperative to consider the different and divergent sources of genetic variation to develop drought-tolerant and high-yielding varieties. Studies on trait–marker associations and biomass allocation will improve the selection efficiency in conventional breeding programs [28][60].

5. Breeding Methodologies and Technologies

5.1. Breeding Wheat for Drought-Tolerance—Conventional Approaches

Conventional breeding involves the creation of genetic variation through sexual recombination of genes from contrasting parents and selecting superior progenies for developing improved varieties. Different selection methods are used in the conventional breeding of self-pollinated crops, including wheat. These include bulk selection, pedigree breeding, pure line selection, and single seed descent (SSD) selection methods, among others. The most widely used selection methods in wheat improvement are the bulk selection method, pedigree method and SSD [46][97][98][99], which are briefly outlined below.

Bulk selection involves hybridisation of parents to produce the F_1 generation. The F_1 is selfed to produce the F_2 generation, and the subsequent generations are harvested in bulk to raise the next generations up to the F_5 [100]. This method is simple, convenient, cost-effective, and relatively easy and does not require pedigree recording. Line selection is performed at the F_6 and the evaluations of lines are performed until F_{10} to F_{11} when the population has become homozygous. During this selection method, natural selection is expected to increase the frequency of superior genotypes and improve genotype adaptability to different environmental conditions such as salinity and drought stress [101][102]. The disadvantages of this method include (1) the longer time required to develop a new variety, (2) unavailability of information on trait(s) inheritance since a progeny test is not mandatory, and (3) loss of superior genotypes due to natural selection [100][103].

Single seed descent involves advancing the breeding generations through the use of a single seed after the initial crosses are performed. With this method, F_2 to F_4 generations are advanced without selection and irrespective of individual plant vigour. Selection is only performed later in the F_5 or F_6 when the population is presumably homozygous. This method requires little space, labour and effort and allows rapid advancement of the next generation by retaining sufficiently large and random samples from F_2 generation. The major advantages of SSD are (1) the homozygosity can be obtained very easily and rapidly (two to three generations per year), (2) it is not affected by natural selection and (3) it is amenable to various selection methods, including speed breeding. The demerits of this method are (1) the selection of plants is based on individual phenotype and not the progeny performance and (2) loss of desirable traits due to selection from a single seed per plant [98].

Pedigree breeding begins with the hybridisation of selected pairs of parents, e.g., "a commercial variety and a genotype chosen based on a particular superior trait". A single plant is selected from the segregating F_2 population. Progeny performance is evaluated with the repeated selections from F_3 to F_6 until the recombined genes are homozygous and the population is homogeneous [98]. This method requires record keeping to track parent–progeny relationships [104]. During pedigree breeding, phenotypic selection is performed in the early generations (F_{3-4}), and yield tests are conducted later (F_5 to F_{10}) when the population has reached adequate homozygosity [99]. According to Allard [104], a new variety may be released for commercial production after testing for five years at least at five representative locations.

The success of any phenotypic selection programmes depends on drought-adaptive and constitutive traits that are highly heritable in the breeding generations. Landraces, breeding lines, synthetics, double haploids (DHs) and recombinant inbred lines (RILs) are largely utilised during drought tolerance breeding [46][105][106]. Integration of drought tolerance genes into the high genetic background is often carried out in conventional breeding. For example, a mapping population was developed from a cross of SeriM82 × Babax, whereby SeriM82 was a high yielding genotype while Babax was a drought-tolerant genotype [46]. Conventional breeding requires several generations of screening to identify contrasting breeding parents and develop stable performing varieties through continuous selection across multiple environmental conditions [103]. Contrasting and target production environments are used to identify superior genotypes with specific or broader adaption [6][10][35][45].

Due to the various limitations associated with phenotypic selection, there is the need for complementary breeding approaches such as marker-assisted selection, genomic selection and genome editing to identify and select superior genotypes. This will enable early generation selection and independent to environmental conditions. The advent of molecular markers and genome editing technologies provides opportunities for phenotyping complex traits, thus reducing labour, time and costs for cultivar development. This will improve selection efficiency in conventional breeding, consequently overcoming the shortcomings of conventional breeding.

5.2. Breeding Wheat for Drought-Tolerance—Genomic Resources

Genomic resources are routinely used to complement phenotypic selection. DNA markers are used for genetic analysis and identify and select superior genotypes heterotic groups, introduce and track genes in the breeding processes. Marker analysis enable to integrate essential traits and genes conferring the adaptation and performance of wheat genotypes, including drought and heat stress conditions. Marker-assisted selection (MAS) will allow efficient selection irrespective of the stage of the plant growth and without the influence of the environment and thus shortening the breeding cycle [107].

Molecular markers such as single nucleotide polymorphisms (SNPs) and diversity array technology (DArT) have revolutionized the application of MAS in wheat breeding programs [108]. Several markers associated with drought-responsive agronomic traits have been identified [6][10][15][109]. However, the genetic control of drought tolerance is complex due to the large number of genes, unstable QTL, epistatic interaction of QTL and the large genome size and the complicated genetic background of wheat [44]. Furthermore, most marker technologies indicate the presence or absence of a gene without detailed information on its expression and effects on a trait [110].

The advent of genomic resources to avail high-density genome-wide genotype-by sequencing (GBS) have allowed genomic prediction and selection of superior genotypes with multi-genetic traits at the early stages of the breeding cycle [111][112]. In wheat, GBS or next-generation genotyping are valuable tools that are widely used to discover SNPs, identify genetic variations, reduce genome complexity and predict genetic gains [112]. Genomic selection (GS) involves the following steps: (1) phenotyping of diverse sets of populations for different quantitative traits and genotyping across the entire genome to predict the performance of the distinct population, (2) genotyping of breeding population to estimate the genomic estimated breeding values (GEBVs), (3) validation of both sets of populations by phenotyping and genotyping and (4) investigation of genetic gains over time [112][113][114]. GS is carried out to accommodate all minor-effect QTL, to identify individuals with the highest GEBVs for target traits and to reduce the number of generations required to select a superior phenotype. The combination of GS with phenotypic selection (PS) showed an improvement of yield by 23%, indicating that genetic gains could be improved by complementing conventional PS with GS in breeding programs [112]. However, GS is yet to be explored for drought tolerance breeding since drought patterns may vary over space and time across wheat varieties and locations.

5.3. Gene editing Technologies and Breeding for Drought Tolerance

The wheat genome is a complex build-up of genes from three genomes (AABBDD, 2n = 6x = 42) [115]. This renders complex regulatory pathways to interact, constitute and maintain genetic homeostasis. Genome editing alters particular genomic regions through insertion, deletion and substitution of genes [115][116][117][118][119]. Thus, any new gene(s) inserted or substituted in the genome should be stable and in a desirable direction [120].

In the past years, genome editing has enabled scientists to generate targeted modifications in organisms of interest [121][122]. The genetic engineering approach efficiency in organisms through genome editing involves nucleases: zinc-finger nucleases (ZFNs), transcription activator-like-effector nucleases (TALENs) and the clustered regularly interspaced short palindromic repeat (CRISPR/Cas systems) [122][123] such as CRISPR/Cas9, CRISPR/sgRNA and CRISPR/Cpf1 [124][125][126][127]. Notably, CRISPR/Cas9 could aid in the rapid improvement of drought and locally adapted varieties by integrating genes from wild relatives showing drought tolerance. This will deliver new commercial varieties that still retain stress resistance traits of their wild relatives [128].

The use of genomic resources and technologies could accelerate molecular breeding and improvement of crops for adaptability to abiotic and biotic stresses. Intra-or inter-genus and species transfer of alien genes for drought and heat tolerance can be deployed successfully to improve wheat adaptability. For example, the transfer of foreign genes modulating stress-adaptive traits, including hormones, dehydration-responsive element-binding proteins (*DREB*), enzymes and deeper rooting genes (*DRO1*) have proved that wheat adaptation and performance can be improved [129]. An example of intragenus transfer of a gene(s) includes *DRO1* and *DREB* genes. *DRO1* controls root growth angle in rice [64][130] and influences the orientation of the root system in wheat [129]. *DREB* are involved in tolerance to numerous abiotic stresses, including drought, salinity, low temperature and abscisic acid (ABA) in wheat [131], and drought tolerance and growth retardation in rice [132].

The application of these technologies is not limited to drought tolerance studies. For example, CRISPR/Cas9 technologies have confirmed their simplicity, proficiency, flexibility and wide adaptability and applicability in several plant-based applications [133]. For example, they have been used for targeted mutagenesis in chickpeas to unravel responsive genes under drought stress [134]. Loss-of-function mutations are the most genomic modification that occurred during the domestication whereby they were stacked in key genes controlling traits such as flowering, seed shattering, colour and size through the application of CRISPR/Cas9 [135]. This has enabled breeders to retrace thousands of years of crop improvement in the process of de novo domestication.

One of the main limiting factors to genome editing is the plant transformation efficiency, which hinders the transfer of edited material into the target cells [128]. The role of genome editing is to change an organism's DNA (add, remove or alter) at a particular location in the genome. Therefore, repairing the pathways of DNA double-strand break (DSB) are homology-dependent repair (HDR) in which a donor sequence matching the target is copied, and nonhomologous end-joining (NHEJ) in which rejoining the broken ends can lead to mutations at the break site [118]. The advent and high potential of genome editing in crops are continuing to drive the development of more effective plant transformation approaches.

5.4. Genes Associated with Drought Tolerance

A gene is considered a candidate when it is associated with a known or proposed function determining the QTL of a trait of interest. As drought brings about changes in gene expression, identification of the expression of candidate genes under

drought stress is important. Wheat genotypes use multiple mechanisms to respond to drought stress and numerous genes condition these mechanisms. This includes genes involved in the coding proteins involved in osmotic adjustment, repairs, transcriptions and regulations [136][137][138][139][140]. Several bio-chemicals assist in regulating dehydration membrane stabilization and osmotic adjustments, among other functions [44][141], thus enabling wheat response to drought at different growth stages [51].

Genes controlling root architecture play a significant role in resource acquisition such as water and nutrients and have been widely targeted in drought tolerance breeding. DEEPER ROOTING1 (*DRO1*) gene, a rice QTL controlling root growth angle reportedly played a significant role in altering root system architecture, thus improving drought avoidance [129][142]. Rice *DRO1* orthologs and wheat *DRO1* orthologs share 76% identity, suggesting the possibility of functional similarity and potential contribution in manipulating root surface area (RSA) for drought avoidance in wheat [140]. Deep-rooted plants contribute to drought avoidance by extracting moisture from deeper soil layers [13]. A field study assessed the effect of drought on changes in wheat transcriptome during the early reproductive stage and discovered 309 differentially expressed genes (*DEGs*) involved in various critical processes such as floral development, photosynthetic activity and stomatal movement [51]. Candidate gene *TaELF3* for earliness per se (Eps) locus has proved to play a significant role in regulating flowering time [143]. Green revolution genes such as *Rht-B1b* and *Rht-D1b* significantly reduce plant height in wheat [144]. *GPC-B1* is an important gene regulating gluten protein content in wheat, thus significantly affecting grain yield [106]. Genes such as *TaSNAC8-6A*, *TaMYB3R1* and *TaNAC69* have been reported to contribute to drought response in wheat [145][146][147]. This represents valuable wheat genetic resources for the improvement of drought tolerance. However, the impact of these genes on grain yield remains to be elucidated. Gene pyramiding and stacking by crossing complementary drought tolerance genotypes/traits from different growth stages could boost drought adaptation and grain yield.

Gene cloning in wheat has been achieved using comparative genomics approaches between wheat and rice, resulting in yield-related genes such as *TaTGW6* [148], *TaGW2-6A* [149] and *TaGS-D1* [150] and *TaSus2* [151] among others. *TaMYBsm3* and *TaCRT1* are among other genes cloned for wheat adaptation to abiotic stresses such as drought [152][153]. The discovery of these genes has provided useful information in understanding the genetics of wheat adaptation to target environments, yield stability, and its contributing traits' performance under such conditions. Such breakthroughs can be utilised in MAS and genomic selection to accelerate breeding, variety development and deployment.

5.5. QTLs Associated with Root and Shoot Attributes under Drought Conditions

Genetic improvement of drought-responsive root attributes and their contribution to higher and stable grain yield through the integration of advanced genomic approaches is of immense importance. Initially, QTL mapping involved the use of bi-parental crosses in different genetic backgrounds. This approach enabled estimation of the number of genomic regions controlling the specific traits in defined populations, characterization of the genomic regions with regard to map position, gene function, phenotypic and pleiotropic effects and epistatic interactions with other QTL. However, this was limited in allelic diversity, genomic resolution and the longer time required to develop mapping populations.

Genome-wide association study (GWAS) and/or marker–trait association (MTA) approach overcomes several QTL mapping limitations by producing higher resolution, based on linkage disequilibrium across the genome, exploiting/employing data from diverse genetic backgrounds, making these approaches more efficient [63][109][154][155]. With GWAS/MTAs, an extensive collection of wheat germplasm is genotyped with SNPs or DArT markers throughout the genome to identify associations with the phenotypic trait(s) of interest [43][109][156][157][158]. However, the large and complex wheat genome, and incompatible genome sequence make GWAS and MTAs studies challenging for identifying genomic regions underlying the observed phenotypes. The availability of wheat sequence reference genome has allowed annotation of functional genes [159], thus enhancing understanding of genome architecture, gene expression, the relationship between drought tolerance genes/QTLs and their conditioning factors [156][157][158][160]. In the past 68 years (1947–2015), multi-trait MTAs or genomic regions have significantly contributed to yield gains of 2.63 to 25.7 million tons [156]. Nearly 800 MTAs/GWAS were reported for drought-responsive traits, i.e., agronomic, physiological, roots and its related traits [139]. Significant MTAs were reported for 36 agro-morphological traits [63].

Mwadzingeni et al. [161] have comprehensively documented genomic regions associated with agro-morphological traits. However, genomic studies on the relationship between root and shoot as well as yield traits remain to be elucidated. This is because root and shoot traits are complex, controlled by polygenes, QTL and environmental effects; therefore, it is challenging to quantify under field conditions. Despite the likely importance of roots and shoots in wheat performance and drought tolerance, few genomic studies on these traits have been undertaken [43][60][160]. Wheat genome B and D [162] have shown to be the main genomes influencing root traits response to drought stress suggesting that these regions are

pleitropic and have multiple genes influencing root development. Wheat genome D contained the most loci for root traits under drought-stressed conditions [15][43]. Kabir et al. [163] found chromosomes 2A, 3A, 4D and 5A in a DH population and chromosomes 3B, 4A, 4D and 5B in RIL population as the main loci influencing root parameters. Chromosome 4D harbours pleiotropic QTL for root traits in DH and RIL populations, while chromosome 3A had the pleiotropic markers in the DH population. In addition to locus 3A, chromosomes 2B and 2D have shown pleitropic QTL for root parameters [164]. QTL on 1B, 3D, 4D, 5A and 5B for coleoptile length were found while chromosome 3D, 4D and 5A showed pleiotropism for plant height [165]. QTL for coleoptile length were found on chromosomes 3B and 4B [166]. These results show that QTLs for root parameters are genetically complex and highly influenced by the growth medium and the plant genotype. Some favourable alleles are not well recorded from different environments and genetic backgrounds, thus presenting opportunities in deploying specific alleles with the use of molecular markers. Therefore, it is important to develop accurate, reliable and well-defined phenotyping assays and techniques to elucidate the mechanisms underlying tolerance to drought stress and high yield under drought-stressed conditions. Identifying genomic regions associated with important breeding traits under rainfed and drought stress conditions and characterization of their genetic make-up is of paramount importance. This will improve the development of breeding pools with positive and beneficial alleles and introgression through MAS. Current and future breeding programs can devise strategies to accumulate these alleles to increase genetic gains.

6. Outlook and conclusion

Wheat breeders and agronomists strive to increase rates of genetic gains for grain yield to support a growing population. Though yield improvement is a key target for most breeding programs globally, current breeding pipelines are not optimised for selecting drought adaptive and constitutive traits and yield. Selection accuracy for quantitative traits may be accelerated by evaluating more selection candidates in multi-environments using well-defined phenotyping assays and phenotyping tools to deliver drought-tolerant varieties. Various selection methods for grain yield and its contributing agro-morphological traits under drought conditions, molecular markers, and genomic regions have been adopted to develop drought-suited varieties. However, simultaneous improvement of drought tolerance and yield through its breeding traits has been perceived as challenging due to inadequate phenotyping techniques, challenges in identifying key breeding traits and large genotype by environment interaction. The large and complex genome size of wheat influencing the expression of multiple genes and QTLs under a given set of conditions makes breeding for quantitative traits complicated. Labour costs, time and space constraints are among major limiting factors.

Strategies such as the cultivation of high-yielding cultivars, high-throughput phenotyping, increased irrigation and water use efficiency are of immense importance. Understanding of environmental variables and agronomic factors determining wheat response to drought and yield is paramount. The exploitation of genetic resources such as landraces, synthetics and wild relatives will lead to the discovery of the wealth of important alleles that can be used in breeding and improvement programs. Advances in molecular markers and marker technologies such as QTL analysis and detection could accelerate genetic selections for significant breeding traits thus reducing the breeding cycle. The availability of wheat sequence reference genome has allowed annotation of functional genes, thus enhancing discovery and understanding of genome architecture, gene expression, the relationship between drought tolerance genes/QTLs and their conditioning factors. Improvement in data analysis techniques will give more power to identify genes and trait associations as well as guide breeders on the yearly status or progress of breeding in their programs. Therefore, elucidation of genetic loci underlying significant breeding traits facilitating wheat adaptation and tolerance to drought and their expression patterns to drought stress will provide a strong foundation for knowledge-based breeding approaches and strategies for improved germplasm for multi-environment and environment-specific niches.

References

1. Tilman, D.; Balzer, C.; Hill, J.; Befort, B.L. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. USA 2011, 108, 20260–20264.

2. Daryanto, S.; Wang, L.; Jacinthe, P.-A. Global synthesis of drought effects on maize and wheat production. PLoS ONE 2016, 11, e0156362.

3. Nalley, L.; Dixon, B.; Chaminuka, P.; Naledzani, Z.; Coale, M.J. The role of public wheat breeding in reducing food insecurity in South Africa. PLoS ONE 2018, 13, e0209598.

4. Khadka, K.; Raizada, M.N.; Navabi, A. Recent progress in germplasm evaluation and gene mapping to enable breeding of drought-tolerant wheat. Front. Plant Sci. 2020, 11, 1149.

5. Bayoumi, T.Y.; Eid, M.H.; Metwali, E.M. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotechnol. 2008, 7, 2341–2352.

6. Afzal, F.; Reddy, B.; Gul, A.; Khalid, M.; Subhani, A.; Shazadi, K.; Quraishi, U.M.; Ibrahim, A.M.; Rasheed, A. Physiological, biochemical and agronomic traits associated with drought tolerance in a synthetic-derived wheat diversity panel. Crop Pasture Sci. 2017, 68, 213–224.

7. Chenu, K.; Deihimfard, R.; Chapman, S.C. Large-scale characterization of drought pattern: A continent-wide modelling approach applied to the Australian wheatbelt-spatial and temporal trends. New Phatol. 2013, 198, 801–820.

8. Sarto, M.V.M.; Sarto, J.R.W.; Rampim, L.; Bassegio, D.; da Costa, P.F.; Inagaki, A.M. Wheat phenology and yield under drought: A review. Aust. J. Crop. Sci. 2017, 11, 941–946.

9. Hoffman, M.T.; Carrick, P.J.; Gillson, L.; West, A.G. Drought, climate change and vegetation response in the succulent karoo, South Africa. S. Afr. J. Sci. 2009, 105, 54–60.

10. Mwadzingeni, L.; Shimelis, H.; Rees, D.J.G.; Tsilo, T.J. Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. PLoS ONE 2017, 12, e0171692.

11. Dolferus, R. To grow or not to grow: A stressful decision for plants. Plant Sci. 2014, 229, 247–261.

12. Aslam, M.; Maqbool, M.A.; Cengiz, R. Mechanisms of drought resistance. In Drought Stress in Maize (Zea mays L.); Springer: Cham, Switzerland, 2015; pp. 19–36.

13. Naeem, M.K.; Ahmad, M.; Kamran, M.; Shah, M.K.N.; Iqbal, M.S. Physiological responses of wheat (Triticum aestivum L.) to drought stress. Int. J. Plant Soil Sci. 2015, 6, 1–9.

14. Shamuyarira, K.W.; Shimelis, H.; Tapera, T.; Tsilo, T.J. Genetic advancement of newly developed wheat populations under drought-stressed and non-stressed conditions. J. Crop Sci. Biotechnol. 2019, 22, 169–176.

15. Ibrahim, S.E.; Schubert, A.; Pillen, K.; Lèon, J. QTL analysis of drought tolerance for seedling root morphological traits in an advanced backcross of spring wheat. Int. J. Agric. Sci. 2012, 2, 619–626.

16. Ren, Y.; He, X.; Liu, D.; Li, J.; Zhao, X.; Li, B.; Tong, Y.; Zhang, A.; Li, Z. Major quantitative trait loci for seminal root morphology of wheat seedlings. Mol. Breed. 2012, 30, 139–148.

17. Bowne, J.B.; Erwin, T.A.; Juttner, J.; Schnurbusch, T.; Langridge, P.; Bacic, A.; Roessner, U. Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. Mol. Plant 2012, 5, 418–429.

18. Dong, Y.; Liu, J.; Geng, H.; Rasheed, A.; Xiao, Y.; Cao, S.; Fu, L.; Yan, J.; Wen, W.; Zhang, Y.; et al. Genome-wide association of stem water soluble carbohydrates in bread wheat. PLoS ONE 2016, 11, e0164293.

19. Shi, S.; Azam, F.I.; Li, H.; Chang, X.; Li, B.; Jing, R. Mapping QTL for stay-green and agronomic traits in wheat under diverse water regimes. Euphytica 2017, 213, 246.

20. Mwadzingeni, L.; Shimelis, H.; Tesfay, S.; Tsilo, T.J. Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. Front. Plant Sci. 2016, 7, 1276.

21. Mkhabela, S.S.; Shimelis, H.; Odindo, A.O.; Mashilo, J. Response of selected drought tolerant wheat (Triticum aestivum L.) genotypes for agronomic traits and biochemical markers under drought-stressed and non-stressed conditions. Acta Agric. Scand. Sect. B-Soil Plant Sci. 2019, 69, 674–689.

22. Mwadzingeni, L.; Shimelis, H.; Tsilo, T.J. Combining ability and gene action controlling yield and yield components in bread wheat (Triticum aestivum L.) under drought-stressed and nonstressed conditions. Plant Breed. 2018, 137, 502–513.

23. Zhang, J.; Zhang, S.; Cheng, M.; Jiang, H.; Zhang, X.; Peng, C.; Lu, X.; Zhang, M.; Jin, J. Effect of drought on agronomic traits of rice and wheat: A meta-analysis. Int. J. Environ. Res. Public Health 2018, 15, 839.

24. Mkhabela, S.S.; Shimelis, H.; Mashilo, J. Genetic differentiation of selected drought and heat tolerant wheat genotypes using simple sequence repeat markers and agronomic traits. S. Afr. J. Plant Soil 2020, 37, 211–219.

25. Praba, M.L.; Cairns, J.E.; Babu, R.C.; Lafitte, H.R. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. J. Agron. Crop Sci. 2009, 195, 30–46.

26. Prasad, G.P.; Pisipati, S.R.; Momčilović, I.; Ristic, Z. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. J. Agron. Crop Sci. 2011, 197, 430–441.

27. Becker, S.R.; Byrne, P.F.; Reid, S.D.; Bauerle, W.L.; McKay, J.K.; Haley, S.D. Root traits contributing to drought tolerance of synthetic hexaploid wheat in a greenhouse study. Euphytica 2016, 207, 213–224.

28. Mathew, I.; Shimelis, H.; Mutema, M.; Clulow, A.; Zengeni, R.; Mbava, N.; Chaplot, V. Selection of wheat genotypes for biomass allocation to improve drought tolerance and carbon sequestration into soils. J. Agron. Crop Sci. 2019, 205,

385–400.

29. Rojas, O.; Vrieling, A.; Rembold, F. Assessing drought probability for agricultural areas in Africa with coarse resolution remote sensing imagery. Remote Sens. Environ. 2011, 115, 343–352.

30. Keneni, G.; Bekele, E.; Imtiaz, M.; Dagne, K.; Alemaw, G. Challenges associated with crop breeding for adaptation to drought-prone environments. Ethiop. J. Agric. Sci. 2016, 27, 1–24.

31. Baloch, M.J.; Dunwell, J.; Dennet, M.; Rajpar, I.; Jatoi, W.A.; Veesar, N.F. Evaluating spring wheat cultivars for drought tolerance through yield and physiological parameters at booting and anthesis. Afr. J. Biotechnol. 2012, 11, 11559–11565.

32. Ayalew, H.; Liu, H.; Börner, A.; Kobiljski, B.; Liu, C.; Yan, G. Genome-wide association mapping of major root length QTLs under PEG induced water stress in wheat. Front. Plant Sci. 2018, 9, 1–9.

33. Farooq, M.; Hussain, M.; Siddique, K.H. Drought stress in wheat during flowering and grain-filling periods. Crit. Rev. Plant Sci. 2014, 33, 331–349.

34. Allahverdiyev, T.I.; Talai, J.M.; Huseynova, I.M.; Aliyev, J.A. Effect of drought stress on some physiological parameters, yield, yield components of durum (Triticum durum desf.) and bread (Triticum aestivum L.) wheat genotypes. Ekin J. Crop Breed. Genet. 2015, 1, 50–62.

35. Iqbal, J. Morphological, physiological and molecular markers for the adaptation of wheat in drought condition. Asian J. Biotechnol. Genet. Eng. 2019, 2, 1–13.

36. Sobhaninan, N.; Heidari, B.; Tahmasebi, S.; Dadkhodale, A.; McIntyre, C.L. Response of quantitative and physiological traits to drought stress in the SeriM82/Babax wheat population. Euphytica 2019, 215, 1–15.

37. Pradhan, G.P.; Prasad, P.V.; Fritz, A.K.; Kirkham, M.B.; Gill, B.S. Effects of drought and high temperature stress on synthetic hexaploid wheat. Funct. Plant Biol. 2012, 39, 190–198.

38. Weldearegay, D.F.; Yan, F.; Jiang, D.; Liu, F. Independent and combined effects of soil warming and drought stress during anthesis on seed set and grain yield in two spring wheat varieties. J. Agron. Crop Sci. 2012, 198, 245–253.

39. Bennett, D.; Reynolds, M.; Mullan, D.; Izanloo, A.; Kuchel, H.; Langridge, P.; Schnurbusch, T. Detection of two major grain yield QTL in bread wheat (Triticum aestivum L.) under heat, drought and high yield potential environments. Theor. Appl. Genet. 2012, 125, 1473–1485.

40. Havrlentová, M.; Kraic, J.; Gregusová, V.; Kovácsová, B. Drought Stress in Cereals—A Review. Agriculture (Pol'nohospodárstvo) 2021, 67, 47–60.

41. Siddiqui, M.N.; Leon, J.; Naz, A.A.; Balivora, A. Genetics and genomics of root system variation in adaptation to drought stress in cereal crops. J. Exp. Bot. 2021, 72, 1007–1019.

42. Abdolshahi, R.; Nazari, M.; Safarian, A.; Sadathossini, T.S.; Salarpour, M.; Amiri, H. Integrated selection criteria for drought tolerance in wheat (Triticum aestivum L.) breeding programs using discriminant analysis. Field Crops Res. 2015, 174, 20–29.

43. Khodaee, S.M.M.; Hashemi, M.; Mirlohi, A.; Majidi, M.M.; Sukumaran, S.; Moghaddam, M.E.; Abdollahi, M. Root characteristics of an elite spring wheat panel under contrasting water treatments and their genome-wide association study. Rhizosphere 2021, 19, 100413.

44. Ashraf, M. Inducing drought tolerance in plants: Recent advances. Biotechnol. Adv. 2010, 28, 169–183.

45. Pinto, R.S.; Reynolds, M.P.; Mathews, K.L.; McIntyre, C.L.; Olivares-Villegas, J.J.; Chapman, S.C. Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. Theor. Appl. Genet. 2010, 121, 1001–1021.

46. Tahmasebi, S.; Heidari, B.; Pakniyat, H.; McIntyre, L. Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (Triticum aestivum L.). Genome 2017, 60, 26–45.

47. Li, L.; Mao, X.; Wang, J.; Chang, X.; Reynolds, M.; Jing, R. Genetic dissection of drought and heat-responsive agronomic traits in wheat. Plant Cell Environ. 2019, 42, 2540–2553.

48. Langridge, P.; Reynolds, M.P. Genomic tools to assist breeding for drought tolerance. Curr. Opin. Biotechnol. 2015, 32, 130–135.

49. Semahegn, Y.; Shimelis, H.; Laing, M.; Mathew, I. Evaluation of bread wheat (Triticum aestivum L.) genotypes for yield and related traits under drought stress conditions. Acta Agric. Scand. Sect. B-Soil Plant Sci. 2020, 70, 474–484.

50. Luo, L.; Xia, H.; Lu, B.-R. Editorial: Crop breeding for drought resistance. Front. Plant Sci. 2019, 10, 314.

51. Ma, J.; Li, R.; Wang, H.; Li, D.; Wang, X.; Zhang, Y.; Zhen, W.; Duan, H.; Yan, G.; Li, Y. Transcriptomics analyses reveal wheat responses to drought stress during reproductive stages under field conditions. Front. Plant Sci. 2017, 8, 592.

52. Guo, X.; Xin, Z.; Yang, T.; Ma, X.; Zhang, Y.; Wang, Z.; Ren, Y.; Lin, T. Metabolomics response for drought stress tolerance in chinese wheat genotypes (Triticum aestivum). Plants 2020, 9, 520.

53. Sun, C.; Ali, K.; Yan, K.; Fiaz, S.; Dormatey, R.; Bi, Z.; Bai, J. Exploration of epigenetics for improvement of drought and other stress resistance in crops: A review. Plants 2021, 10, 1226.

54. Nezhad, K.Z.; Weber, W.E.; Röder, M.S.; Sharma, S.; Lohwasser, U.; Meyer, R.C.; Saal, B.; Börner, A. QTL analysis for thousand-kernel weight under terminal drought stress in bread wheat (Triticum aestivum L.). Euphytica 2012, 186, 127–138.

55. Shavrukov, Y.; Kurishbayev, A.; Jatayev, S.; Shvidchenko, V.; Zotova, L.; Koekemoer, F.; De Groot, S.; Soole, K.; Langridge, P. Early flowering as a drought escape mechanism in plants: How can it aid wheat production? Front. Plant Sci. 2017, 8, 1–8.

56. Kashiwagi, J.; Krishnamurthy, L.; Upadhyaya, H.D.; Krishna, H.; Chandra, S.; Vadez, V.; Serraj, R. Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (Cicer arietinum L.). Euphytica 2005, 146, 213–222.

57. Kapoor, D.; Bhardwaj, S.; Landi, M.; Sharma, A.; Ramakrishnan, M.; Sharma, A. The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Appl. Sci. 2020, 10, 5692.

58. Monneveux, P.; Jing, R.; Misra, S. Phenotyping for drought adaptation in wheat using physiological traits. Front. Physiol. 2012, 3, 429.

59. Farzamipour, M.R.; Moghaddam, M.; Aharizad, S.; Rashidi, V. Genetic variation for agronomic characters and drought tolerance among the recombinant inbred lines of wheat from the Norstar × Zagross cross. Int. J. Biosci. 2013, 3, 76–86.

60. Mathew, I.; Shimelis, H.; Shayanowako, A.I.T.; Laing, M.; Chaplot, V. Genome-wide association study of drought tolerance and biomass allocation in wheat. PLoS ONE 2019, 14, e0225383.

61. Farshadfar, E.; Jamshidi, B.; Aghaee, M. Biplot analysis of drought tolerance indicators in bread wheat landraces of Iran. Int. J. Agric. Crop Sci. 2012, 4, 226–233.

62. Bilal, M.; Rashid, R.M.; Rehman, S.U.; Iqbal, F.; Ahmed, J.; Abid, M.A.; Ahmed, Z.; Hayat, A. Evaluation of wheat genotypes for drought tolerance. J. Green Physiol. Genet. Genom. 2015, 1, 11–21.

63. Sheoran, S.; Jaiswal, S.; Kumar, D.; Raghav, N.; Sharma, R.; Pawar, S.; Paul, S.; Iquebal, M.A.; Jaiswar, A.; Sharma, P.; et al. Uncovering genomic regions associated with 36 agro-morphological traits in Indian spring wheat using GWAS. Front. Plant Sci. 2019, 10, 1–20.

64. Sallam, A.; Alqudah, A.M.; Dawood, M.F.; Baenziger, P.S.; Börner, A. Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. Int. J. Mol. Sci. 2019, 20, 3137.

65. Christopher, J.T.; Christopher, M.J.; Borrell, A.K.; Fletcher, S.; Chenu, K. Stay-green traits to improve wheat adaptation in well-watered and water-limited environments. J. Exp. Bot. 2016, 67, 5159–5172.

66. Calderini, D.F.; Reynolds, M.P. Changes in grain weight as a consequence of de-graining treatments at pre-and post-anthesis in synthetic hexaploid lines of wheat (Triticum durum x T. tauschii). Funct. Plant Biol. 2000, 27, 183–191.

67. Chen, J.; Liang, Y.; Hu, X.; Wang, X.; Tan, F.; Zhang, H.; Ren, Z.; Luo, P. Physiological characterization of 'stay green'wheat cultivars during the grain filling stage under field growing conditions. Acta Physiol. Plant. 2010, 32, 875–882.

68. Christopher, J.T.; Manschadi, A.M.; Hammer, G.L.; Borrell, A.K. Developmental and physiological traits associated with high yield and stay-green phenotype in wheat. Aust. J. Agric. Res. 2008, 59, 354–364.

69. Babar, M.A.; Reynolds, M.P.; Van Ginkel, M.; Klatt, A.R.; Raun, W.R.; Stone, M.L. Spectral reflectance to estimate genetic variation for in-season biomass, leaf chlorophyll, and canopy temperature in wheat. Crop Sci. 2006, 46, 1046–1057.

70. Lopes, M.S.; Reynolds, M.P. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct. Plant Biol. 2010, 37, 147–156.

71. Marcińska, I.; Czyczyło-Mysza, I.; Skrzypek, E.; Filek, M.; Grzesiak, S.; Grzesiak, M.T.; Janowiak, F.; Hura, T.; Dziurka, M.; Dziurka, K.; et al. Impact of osmotic stress on physiological and biochemical characteristics in drought-susceptible and drought-resistant wheat genotypes. Acta Physiol. Plant. 2013, 35, 451–461.

72. Lawlor, D.W.; Cornic, G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 2002, 25, 275–294.

73. Li, M.; Liu, Y.; Ma, J.; Zhang, P.; Wang, C.; Su, J.; Yang, D. Genetic dissection of stem WSC accumulation and remobilization in wheat (Triticum aestivum L.) under terminal drought stress. BMC Genet. 2020, 21, 50.

74. Javadi, T.; Arzani, K.; Ebrahimzadeh, H. Study of proline, soluble sugar, and chlorophyll a and b changes in nine Asian and one European pear cultivar under drought stress. In Proceedings of the XXVII International Horticultural Congress-IHC2006: International Symposium on Asian Plants with Unique Horticultural, Seoul, Korea, 30 June 2008; Volume 769, pp. 241–246.

75. Borrill, P.; Fahy, B.; Smith, A.M.; Uauy, C. Wheat grain filling is limited by grain filling capacity rather than the duration of flag leaf photosynthesis: A case study us-ing NAM RNAi plants. PLoS ONE 2015, 10, e0134947.

76. Hassan, N.M.; El-Bastawisy, Z.M.; El-Sayed, A.K.; Ebeed, H.T.; Alla, M.M.N. Roles of dehydrin genes in wheat tolerance to drought stress. J. Adv. Res. 2015, 6, 179–188.

77. Islam, M.; Begum, M.C.; Kabir, A.H.; Alam, M.F. Molecular and biochemical mechanisms associated with differential responses to drought tolerance in wheat (Triticum aestivum L.). J. Plant Interact. 2015, 10, 195–201.

78. Mathew, I.; Shimelis, H.; Mwadzingeni, L.; Zengeni, R.; Mutema, M.; Chaplot, V. Variance components and heritability of traits related to root: Shoot biomass allocation and drought tolerance in wheat. Euphytica 2018, 214, 225.

79. Ober, E.S.; Alahmad, S.; Cockram, J.; Forestan, C.; Hickey, L.T.; Kant, J.; Maccaferri, M.; Marr, E.; Milner, M.; Pinto, F.; et al. Wheat root systems as a breeding target for climate resilience. Theor. Appl. Genet. 2021, 134, 1645–1662.

80. Wasson, A.P.; Richards, R.A.; Chatrath, R.; Misra, S.C.; Prasad, S.S.; Rebetzke, G.J.; Kirkegaard, J.A.; Christopher, J.; Watt, M. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J. Exp. Bot. 2012, 63, 3485–3498.

81. Peng, B.; Liu, X.; Dong, X.; Xue, Q.; Neely, C.B.; Marek, T.; Ibrahim, A.M.; Zhang, G.; Leskovar, D.I.; Rudd, J.C. Root morphological traits of winter wheat under contrasting environments. J. Agron. Crop Sci. 2019, 205, 571–585.

82. Meister, R.; Rajani, M.S.; Ruzicka, D.; Schachtman, D.P. Challenges of modifying root traits in crops for agriculture. Trends Plant Sci. 2014, 19, 779–788.

83. Faye, A.; Sine, B.; Chopart, J.L.; Grondin, A.; Lucas, M.; Diedhiou, A.G.; Gantet, P.; Cournac, L.; Min, D.; Audebert, A.; et al. Development of a model estimating root length density from root impacts on a soil profile in pearl millet (Pennisetum glaucum (L.) R. Br). Application to measure root system response to water stress in field conditions. PLoS ONE 2019, 14, e0214182.

84. Eissenstat, D.M.; Kucharski, J.M.; Zadworny, M.; Adams, T.S.; Koide, R.T. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. New Phytol. 2015, 208, 114–124.

85. El-Hassouni, K.; Alahmad, S.; Belkadi, B.; Filali-Maltouf, A.; Hickey, L.T.; Bassi, F.M. Root system architecture and its association with yield under different water regimes in durum wheat. Crop Sci. 2018, 58, 2331–2346.

86. Kamal, N.M.; Gorafi, Y.S.A.; Abdelrahman, M.; Abdellatef, E.; Tsujimoto, H. Stay-green trait: A prospective approach for yield potential, and drought and heat stress adaptation in globally important cereals. Int. J. Mol. Sci. 2019, 20, 5837.

87. Schachtman, D.P.; Goodger, J.Q. Chemical root to shoot signaling under drought. Trends Plant Sci. 2008, 13, 281–287.

88. Bengough, A.G.; McKenzie, B.M.; Hallett, P.D.; Valentine, T.A. Root elongation, water stress, and mechanical impedance: A review of limiting stresses and beneficial root tip traits. J. Exp. Bot. 2011, 62, 59–68.

89. Hunt, J.R.; Lilley, J.M.; Trevaskis, B.; Flohr, B.M.; Peake, A.; Fletcher, A.; Zwart, A.B.; Gobbett, D.; Kirkegaard, J.A. Early sowing systems can boost Australian wheat yields despite recent climate change. Nat. Clim. Chang. 2019, 9, 244–247.

90. Gao, S.Q.; Chen, M.; Xu, Z.S.; Zhao, C.P.; Li, L.; Xu, H.J.; Tang, Y.M.; Zhao, X.; Ma, Y.Z. The soybean GmbZIP1 transcription factor enhances multiple abiotic stress tolerances in transgenic plants. Plant Mol. Biol. 2011, 75, 537–553.

91. Fletcher, A.; Chenu, K. Change in biomass partitioning and transpiration efficiency in Australian wheat varieties over the last decades. In Proceedings of the 17th Australian Agronomy Conference, Hobart, Australia, 21–24 September 2015; Volume 4.

92. Fletcher, A.; Chenu, K. Recent Improvements in Biomass Partitioning and Transpiration Efficiency of Modern Australian Wheat Varieties–Any Opportunity for the Future? 2016. Available online: https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2016/03/recent-improvements-in-biomass-partitioning-and-transpiration-efficiency-of-wheat (accessed on 13 February 2022).

93. Kirkegaard, J.A.; Lilley, J.M.; Howe, G.N.; Graham, J.M. Impact of subsoil water use on wheat yield. Aust. J. Agric. Res. 2007, 58, 303–315.

94. Deng, W.; Casao, M.C.; Wang, P.; Sato, K.; Hayes, P.M.; Finnegan, E.J.; Trevaskis, B. Direct links between the vernalization response and other key traits of cereal crops. Nat. Commun. 2015, 6, 5882.

95. Voss-Fels, K.P.; Robinson, H.; Mudge, S.R.; Richard, C.; Newman, S.; Wittkop, B.; Stahl, A.; Friedt, W.; Frisch, M.; Gabur, I.; et al. VERNALIZATION1 modulates root system architecture in wheat and barley. Mol. Plant 2018, 11, 226–

229.

96. Ghimire, B. In Situ Imaging of Root System Architecture to Improve Drought Tolerance and Yield in Spring Wheat (Triticum aestivum L.). Doctoral dissertation, Washington State University: Vancouver, WA, USA, 2017; 177p.

97. Tahmasebi, S.; Heidari, B.; Pakniyat, H.; Dadkhodaie, A. Consequences of 1BL/1RS translocation on agronomic and physiological traits in wheat. Cereal Res. Commun. 2015, 43, 554–566.

98. Breseghello, F.; Coelho, A.S.G. Traditional and modern plant breeding methods with examples in rice (Oryza sativa L.). J. Agric. Food Chem. 2013, 61, 8277–8286.

99. Xynias, I.N.; Mylonas, I.; Korpetis, E.G.; Ninou, E.; Tsaballa, A.; Avdikos, I.D.; Mavromatis, A.G. Durum wheat breeding in the Mediterranean region: Current status and future prospects. Agronomy 2020, 10, 432.

100. Acquaah, G. Principles of Plant Genetics and Breeding, 2nd ed.; Wiley-Blackwell: Oxford, UK, 2012; 740p.

101. Al-Azab, K.F. Improving Wheat for Drought Tolerance by Using Hybridization and Mutation Breeding Procedures. Doctoral Dissertation, Faculty of Agriculture Cairo University, Giza, Egypt, 2013; 266p.

102. Farag, H.I.A. Efficiency of three methods of selection in wheat breeding under saline stress conditions. J. Plant Breed. 2013, 17, 85–95.

103. Acquaah, G. Principles of Plant Genetics and Breeding, 1st ed.; John Wiley & Sons: Hoboken, NJ, USA, 2009; 584p.

104. Allard, R.W. "Plant Breeding". Encyclopedia Britannica. 2019. Available online: https://www.britannica.com/science/plant-breeding (accessed on 3 March 2022).

105. Mwadzingeni, L.; Figlan, S.; Shimelis, H.; Mondal, S.; Tsilo, T. Genetic resources and breeding methodologies for improving drought tolerance in wheat. J. Crop Improv. 2017, 31, 648–672.

106. Distelfeld, A.; Uauy, C.; Fahima, T.; Dubcovsky, J. Physical map of the wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytol. 2006, 169, 753–763.

107. Khan, M.A.; Iqbal, M.; Jameel, M.; Nazeer, W.; Shakir, S.; Aslam, M.T.; Iqbal, B. Potentials of molecular based breeding to enhance drought tolerance in wheat (Triticum aestivum L.). Afr. J. Biotechnol. 2011, 10, 11340–11344.

108. Collard, B.C.; Mackill, D.J. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philos. Trans. R. Soc. B Biol. Sci. 2008, 363, 557–572.

109. Edae, E.A.; Byrne, P.F.; Haley, S.D.; Lopes, M.S.; Reynolds, M.P. Genome-wide association mapping of yield and yield components of spring wheat under contrasting moisture regimes. Theor. Appl. Genet. 2014, 127, 791–807.

110. Huseynova, I.M.; Rustamova, S.M. Screening for drought stress tolerance in wheat genotypes using molecular markers. Proc. ANAS 2010, 65, 132–139.

111. Tessema, B.B.; Liu, H.; Sørensen, A.C.; Andersen, J.R.; Jensen, J. Strategies using genomic selection to increase genetic gain in breeding programs for wheat. Front. Genet. 2020, 11, 1–12.

112. Lozada, D.N.; Ward, B.P.; Carter, A.H. Gains through selection for grain yield in a winter wheat breeding program. PLoS ONE 2020, 15, e0221603.

113. Tadesse, W.; Sanchez-Garcia, M.; Assefa, S.G.; Amri, A.; Bishaw, Z.; Ogbonnaya, F.C.; Baum, M. Genetic gains in wheat breeding and its role in feeding the world. Crop Breed. Genet. Genome 2019, 1, 1–28.

114. Tsai, H.Y.; Janss, L.L.; Andersen, J.R.; Orabi, J.; Jensen, J.D.; Jahoor, A.; Jensen, J. Genomic prediction and GWAS of yield, quality and disease-related traits in spring barley and winter wheat. Sci. Rep. 2020, 10, 3347.

115. Gill, B.S.; Appels, R.; Botha-Oberholster, A.M.; Buell, C.R.; Bennetzen, J.L.; Chalhoub, B.; Chumley, F.; Dvorák, J.; Iwanaga, M.; Keller, B.; et al. A workshop report on wheat genome sequencing: International Genome Research on Wheat Consortium. Genetics 2004, 168, 1087–1096.

116. Sticklen, M. Transgenic, cisgenic, intragenic and subgenic crops. Adv. Crop Sci. Technol. 2015, 3, e123.

117. Puchta, H. Using CRISPR/Cas in three dimensions: Towards synthetic plant genomes, transcriptomes and epigenomes. Plant J. 2016, 87, 5–15.

118. Carroll, D. Focus: Genome editing: Genome editing: Past, present and future. Yale J. Biol. Med. 2017, 90, 653–659.

119. Khan, M.Z.; Zaidi, S.S.E.A.; Amin, I.; Mansoor, S. A CRISPR way for fast-forward crop domestication. Trends Plant Sci. 2019, 24, 293–296.

120. Hansen, M.K. Genetic Engineering Is Not an Extension of Conventional Plant Breeding; How Genetic Engineering Differs from Conventional Breeding, Hybridization, Wide Crosses and Horizontal Gene Transfer; Consumer Policy Institute/Consumers Union: New York, NY, USA, 2000; pp. 1–15.

121. Carroll, D. Genome engineering with zinc-finger nucleases. Genetics 2011, 188, 773–782.

122. Gaj, T.; Gersbach, C.A.; Barba, I.I.I.; Carlos, F. ZFN, TALEN and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 2013, 31, 397–405.

123. Weinthal, D.M.; Gürel, F. Plant genome editing and its applications in cereals. In Genetic Engineering: An Insight into the Strategies and Applications; IntechOpen: London, UK, 2016; pp. 63–73.

124. Belhaj, K.; Chaparro-Garcia, A.; Kamoun, S.; Nekrasov, V. Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 2013, 9, 39.

125. Mali, P.; Aach, J.; Stranges, P.B.; Esvelt, K.M.; Moosburner, M.; Kosuri, S.; Yang, L.; Church, G.M. CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat. Biotechnol. 2013, 31, 833–838.

126. Tsai, S.Q.; Wyvekens, N.; Khayter, C.; Foden, J.A.; Thapar, V.; Reyon, D.; Goodwin, M.J.; Aryee, M.J.; Joung, J.K. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. Nat. Biotechnol. 2014, 32, 569–576.

127. Zetsche, B.; Gootenberg, J.S.; Abudayyeh, O.O.; Slaymaker, I.M.; Makarova, K.S.; Essletzbichler, P.; Volz, S.E.; Joung, J.; Van Der Oost, J.; Re`gev, A.; et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 2015, 163, 759–771.

128. Martignago, D.; Rico-Medina, A.; Blasco-Escámez, D.; Fontanet-Manzaneque, J.B.; Caño-Delgado, A.I. Drought resistance by engineering plant tissue-specific responses. Front. Plant Sci. 2020, 10, 1–19.

129. Ashraf, A.; Rehman, O.U.; Muzammil, S.; Léon, J.; Naz, A.A.; Rasool, F.; Ali, G.M.; Zafar, Y.; Khan, M.R. Evolution of Deeper Rooting 1-like homoeologs in wheat entails the C-terminus mutations as well as gain and loss of auxin response elements. PLoS ONE 2019, 14, e0214145.

130. Guseman, J.M.; Webb, K.; Srinivasan, C.; Dardick, C. DRO 1 influences root system architecture in Arabidopsis and Prunus species. Plant J. 2017, 89, 1093–1105.

131. Wei, B.; Jing, R.; Wang, C.; Chen, J.; Mao, X.; Chang, X.; Jia, J. Dreb1 genes in wheat (Triticum aestivum L.): Development of functional markers and gene mapping based on SNPs. Mol. Breed. 2009, 23, 13–22.

132. Moon, S.J.; Min, M.K.; Kim, J.; Kim, D.Y.; Yoon, I.S.; Kwon, T.R.; Byun, M.O.; Kim, B.G. Ectopic expression of OsDREB1G, a member of the OsDREB1 subfamily, confers cold stress tolerance in rice. Front. Plant Sci. 2019, 10, 1–11.

133. Joshi, R.K.; Bharat, S.S.; Mishra, R. Engineering drought tolerance in plants through CRISPR/Cas genome editing. 3 Biotech 2020, 10, 400.

134. Badhan, S.; Ball, A.S.; Mantri, N. First report of CRISPR/Cas9 mediated DNA-free editing of 4CL and RVE7 genes in chickpea protoplasts. Int. J. Mol. Sci. 2021, 22, 396.

135. Meyer, R.S.; Purugganan, M.D. Evolution of crop species: Genetics of domestication and diversification. Nat. Rev. Genet. 2013, 14, 840–852.

136. Budak, H.; Hussain, B.; Khan, Z.; Ozturk, N.Z.; Ullah, N. From genetics to functional genomics: Improvement in drought signaling and tolerance in wheat. Front. Plant Sci. 2015, 6, 1012.

137. Roy, S.J.; Tucker, E.J.; Tester, M. Genetic analysis of abiotic stress tolerance in crops. Curr. Opin. Plant Biol. 2011, 14, 232–239.

138. Goel, S.; Signh, K.; Grewal, S.; Nath, M. Impact of ¨omics¨ in improving drought tolerance in wheat. Crit. Rev. Plant Sci. 2020, 39, 222–235.

139. Gupta, P.K.; Balyan, H.S.; Gahlaut, V. QTL analysis for drought-tolerance in wheat: Present status and future possibilities. Agronomy 2017, 7, 5.

140. Kulkarni, M.; Soolanayakanahally, R.; Ogawa, S.; Uga, Y.; Selvaraj, M.G.; Kagale, S. Drought response in wheat: Key genes and regulatory mechanisms controlling root system architecture and transpiration efficiency. Front. Chem. 2017, 5, 106.

141. Yang, S.; Vanderbeld, B.; Wan, J.; Huang, Y. Narrowing down the targets: Towards successful genetic engineering of drought-tolerance crops. Mol. Plant 2010, 3, 469–490.

142. Uga, Y.; Okuno, K.; Yano, M. Dro1, a major QTL involved in deep rooting of rice under upland field conditions. J. Exp. Bot. 2011, 62, 2485–2494.

143. Wang, J.; Wen, W.; Hanif, M.; Xia, X.; Wang, H.; Liu, S.; Liu, J.; Yang, L.; Cao, S.; He, Z. TaELF3-1DL, a homolog of ELF3, is associated with heading date in bread wheat. Mol. Breed. 2016, 36, 161.

144. Zhang, J.; Dell, B.; Biddulph, B.; Drake-Brockman, F.; Walker, E.; Khan, N.; Wong, D.; Hayden, M.; Appels, R. Wild-type alleles of Rht-B1 and Rht-D1 as independent determinants of thousand-grain weight and kernel number per spike in wheat. Mol. Breed. 2013, 32, 771–783.

145. Xue, G.G.; Way, H.H.; Richardson, T.; Drenth, J.; Joyce, P.A.; McIntyre, C.L. Overexpression of TaNAC69 leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol. Plant 2011, 4, 697–712.

146. Cai, H.; Tian, S.; Liu, C.; Dong, H. Identification of a MYB3R gene involved in drought, salt and cold stress in wheat (Triticum aestivum L.). Gene 2011, 485, 14–152.

147. Mao, H.; Li, S.; Wang, Z.; Cheng, X.; Li, F.; Mei, F.; Chen, N.; Kang, Z. Regulatory changes in TaSNAC8-6A are associated with drought tolerance in wheat seedlings. Plant Biotechnol. J. 2020, 18, 1078–1092.

148. Hu, M.J.; Zhang, H.P.; Cao, J.J.; Zhu, X.F.; Wang, S.X.; Jiang, H.; Wu, Z.Y.; Lu, J.; Chang, C.; Sun, G.L.; et al. Characterization of an IAA-glucose hydrolase gene TaTGW6 associated with grain weight in common wheat (Triticum aestivum L.). Mol. Breed. 2016, 36, 25.

149. Qin, L.; Zhao, J.; Li, T.; Hou, J.; Zhang, X.; Hao, C. TaGW2, a good reflection of wheat polyploidization and evolution. Front. Plant Sci. 2017, 8, 1–13.

150. Zhang, Y.; Liu, J.; Xia, X.; He, Z. TaGS-D1, an ortholog of rice OsGS3, is associated with grain weight and grain length in common wheat. Mol. Breed. 2014, 34, 1097–1107.

151. Jiang, Q.; Hou, J.; Hao, C.; Wang, L.; Ge, H.; Dong, Y.; Zhang, X. The wheat (T. aestivum) sucrose synthase 2 gene (TaSus2) active in endosperm development is associated with yield traits. Funct. Integr. Genom. 2011, 11, 49–61.

152. An, Y.Q.; Lin, R.M.; Wang, F.T.; Feng, J.; Xu, Y.F.; Xu, S.C. Molecular cloning of a new wheat calreticulin gene TaCRT1 and expression analysis in plant defense responses and abiotic stress resistance. Genet. Mol. Res. 2011, 10, 3576–3585.

153. Li, Y.; Zhang, S.; Zhang, N.; Zhang, W.; Li, M.; Liu, B.; Shi, Z. MYB-CC transcription factor, TaMYBsm3, cloned from wheat is involved in drought tolerance. BMC Plant Biol. 2019, 19, 143.

154. Abou-Elwafa, S.F.; Shehzad, T. Genetic diversity, GWAS and prediction for drought and terminal heat stress tolerance in bread wheat (Triticum aestivum L.). Genet. Resour. Crop Evol. 2021, 68, 711–728.

155. Gizaw, S.A.; Godoy, J.G.V.; Garland-Campbell, K.; Carter, A.H. Genome-wide association study of yield and component traits in Pacific Northwest winter wheat. Crop Sci. 2018, 58, 2315–2330.

156. Ain, Q.-U.; Rasheed, A.; Anwar, A.; Mahmood, T.; Imtiaz, M.; Mahmood, T.; Xia, X.; He, Z.; Quraishi, U.M. Genome-wide association for grain yield under rainfed conditions in historical wheat cultivars from Pakistan. Front. Plant Sci. 2015, 6, 743.

157. Shokat, S.; Sehgal, D.; Vikram, P.; Liu, F.; Singh, S. Molecular markers associated with agro-physiological traits under terminal drought conditions in bread wheat. Int. J. Mol. Sci. 2020, 21, 3156.

158. Rahimi, Y.; Bihamta, M.R.; Taleei, A.; Alipour, H.; Ingvarsson, P.K. Genome-wide association study of agronomic traits in bread wheat reveals novel putative alleles for future breeding programs. BMC Plant Biol. 2019, 19, 541.

159. IWGSC Website. 2018. Available online: http://www.wheatgenome.org (accessed on 19 September 2021).

160. Li, L.; Peng, Z.; Mao, X.; Wang, J.; Chang, X.; Reynolds, M.; Jing, R. Genome-wide association study reveals genomic regions controlling root and shoot traits at late growth stages in wheat. J. Exp. Bot. 2019, 124, 993–1006.

161. Mwadzingeni, L.; Shimelis, H.; Dube, E.; Laing, M.D.; Tsilo, T.J. Breeding wheat for drought tolerance: Progress and technologies. J. Integr. Agric. 2016, 15, 935–943.

162. Liu, X.; Li, R.; Chnag, X.; Jing, R. Mapping QTLs for seedling root traits in a double haploid wheat population under different water regimes. Euphytica 2013, 189, 51–66.

163. Kabir, M.R.; Liu, G.; Guan, P.; Wang, F.; Khan, A.A.; Ni, Z.; Yao, Y.; Hu, Z.; Xin, M.; Peng, H.; et al. Mapping QTLs associated with root traits using two different populations in wheat (Triticum aestivum L.). Euphytica 2015, 206, 175–190.

164. Liu, R.X.; Wu, F.K.; Xin, Y.I.; Yu, L.N.; Wang, Z.Q.; Liu, S.H.; Mei, D.E.N.G.; Jian, M.A.; Wei, Y.M.; Zheng, Y.L.; et al. Quantitative trait loci analysis for root traits in synthetic hexaploid wheat under drought stress conditions. J. Integr. Agric. 2020, 19, 1947–1960.

165. Yu, J.B.; Bai, G.H. Mapping quantitative trait loci for long coleoptile in Chinese wheat landrace Wangshuibai. Crop Sci. 2010, 50, 43–50.

166. Singh, K.; Shukla, S.; Kadam, S.; Semwal, V.K.; Singh, N.K.; Khanna-Chopra, R. Genomic regions and underlying candidate genes associated with coleoptile length under deep sowing conditions in a wheat RIL population. J. Plant Biochem. Biotechnol. 2015, 24, 324–330.