

Review

QTL Analysis for Drought Tolerance in Wheat: Present Status and Future Possibilities

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Abstract: In recent years, with climate change, drought stress has been witnessed in many parts of the world. In many irrigated regions also, shortage of water supply allows only limited irrigation. These conditions have an adverse effect on the productivity of many crops including cereals such as wheat. Therefore, genetics of drought/water stress tolerance in different crops has become a priority area of research. This research mainly involves use of quantitative trait locus (QTL) analysis (involving both interval mapping and association mapping) for traits that are related to water-use efficiency. In this article, we briefly review the available literature on QTL analyses in wheat for traits, which respond to drought/water stress. The outlook for future research in this area and the possible approaches for utilizing the available information on genetics of drought tolerance for wheat breeding are also discussed.

Keywords: morphological traits; physiological traits; drought; genes; quantitative trait loci (QTLs); meta-QTLs; epistatic QTLs; wheat

1. Introduction

Abiotic and biotic stresses are responsible for major losses in crop productivity worldwide. While sufficient information is available about the genetics of resistance against major diseases in all important crops, information about the genetics of tolerance against abiotic stresses is still being collected. Additionally, among all abiotic stresses, drought (water stress) is the single most important stress leading to maximum reduction in crop productivity [1]. Irregular and unpredictable rainfall caused by climate change is likely to further aggravate water stress leading to a decline in productivity of all cereals including wheat in many areas around the world [2,3].

According to some estimates, almost 50% of wheat cultivated in the developing world (50 million ha) is sown under rain-fed systems, which receive less than 600 mm of precipitation per annum. This rainfall could be as low as less than 350 mm per annum in areas inhabited by the poorest and most disadvantaged farmers of the developing countries [4]. The non-availability of adequate water for irrigation as a result of receding water tables [5] is also negatively impacting wheat production in some areas. For instance, it is estimated that although 80% of the wheat crop in India is cultivated under irrigated conditions [6], ~66% of the crop receives only partial irrigation [1,2], subjecting the wheat crop to water stress, and resulting in reduced grain yield [7]. Therefore, there is also a need for breeding wheat cultivars that require partial as opposed to full irrigation.

It is known that most of the traits associated with improved performance under water-limited environments are complex and polygenic in nature (for details, see reviews by Gupta et al. [8] and Farooq et al. [9]). Fortunately, significant genetic variation for traits associated with drought tolerance seems to be available in wheat germplasm [7,10]. Therefore, it will be useful to deploy marker-assisted selection (MAS) involving the available QTLs for drought-related traits for the development of pre-bred

wheat material with improved tolerance to drought stress. For this purpose, elite and high yielding wheat cultivars that are sensitive to drought may be used as recipient parents in a backcrossing program involving a drought-tolerant genotype as the donor parent.

In view of the above, research involving phenotyping, genetics, and breeding for tolerance against drought is currently receiving worldwide attention. For instance, the Consultative Group on International Agriculture Research (CGIAR) program on wheat (CRP WHEAT) established a multi-disciplinary partnership to capture global expertise and resources, tentatively named the Heat and Drought Wheat Improvement Consortium–HeDWIC. Under this program, research ideas that may lead to the development of heat and drought-tolerant wheat genotypes were also invited in July 2014. A global Wheat Yield Consortium (WYC) has also been constituted to address the problem of productivity under abiotic stresses such as drought and heat [11,12]. Several physiological parameters have also become available to allow precise and efficient selection of drought-tolerant genotypes. The national and international status of wheat research in these areas has been reviewed [8,9].

A number of studies involving QTL interval mapping and genome wide association studies (GWAS) have already been conducted in wheat. As a result, a large number of QTLs have already been reported for several traits related to drought tolerance. These traits include coleoptile length, CID or $\Delta^{13}\text{C}$, water-soluble carbohydrates (WSC), root system, grain yield, and related traits recorded under water stress (for a review, see Gupta et al. [8]). Some QTLs for each of these individual traits contribute as much as >20% phenotypic variation. A number of these QTLs are also now being deployed for developing drought-tolerant wheat cultivars (for reviews, see [8,13,14]).

Recent developments in genomics and phenomics allow us more accurate and comprehensive characterization of the QTLs that regulate a particular trait (also known as QTLome). However, the level of information on QTLome is enormous, and approaches to synthesize and translate this information by the breeders needs to be refined. Improved QTL meta-analyses, better estimation of QTL effects and improved crop modeling will enable a more effective exploitation of the QTLome [15]. In this brief review, an effort has been made to review the literature on QTL mapping for drought tolerance in wheat. Future possibilities of conducting genetic studies and utilizing the available information for developing wheat cultivars that are relatively drought-tolerant have also been discussed.

2. Methodology Used for Collecting Literature and Selecting QTLs

The authors have been engaged in research on QTL analysis for almost two decades now and, in recent years, started work on genetics of abiotic stresses including drought and heat tolerance. As a part of this activity, the authors earlier also wrote a review on drought and heat tolerance in wheat [8]; we believe that this review was quite comprehensive and must have covered all the literature available by 2012. Subsequently, other small reviews were also published on QTL analysis for drought tolerance in wheat [13,14,16]. For the present review, the authors compiled and reviewed the information that was available in as many as more than 50 original studies, of which ~20 were published following the publication of their earlier review [8]. A search for research involving QTL analyses for drought tolerance in wheat was undertaken following standard methods of literature search, including both primary and secondary sources. The web resources and various databases were also used for this purpose. To the best of our knowledge, all the published information on QTL analyses under drought (water stress) in wheat has been reviewed in the present article.

3. Morphological, Growth, and Agronomic Responses

When plants are subjected to an abiotic stress such as drought, a diverse set of physiological, metabolic, and defense systems are activated to allow plants to survive and to sustain growth and productivity [17]. Genetics of drought tolerance/sensitivity is complex, and the associated traits are complex and polygenic, thus making the task of developing drought-tolerant cultivars difficult. However, transcriptomics, proteomics, and gene expression studies have allowed identification of the

factors involved in regulation of the synthesis of several proteins, which may provide stress tolerance. Plants being sessile by nature have a system to perceive and respond to conditions such as drought. The perceived signal is transduced and leads to the expression of genes encoding proteins that are involved in providing drought tolerance (Figure 1) [18]. Adaptation to drought by plants is achieved using two different mechanisms including drought avoidance and drought tolerance, which are not mutually exclusive. Different morphological and physiological traits that are involved in each of the two mechanisms for drought adaptation are listed in Table 1.

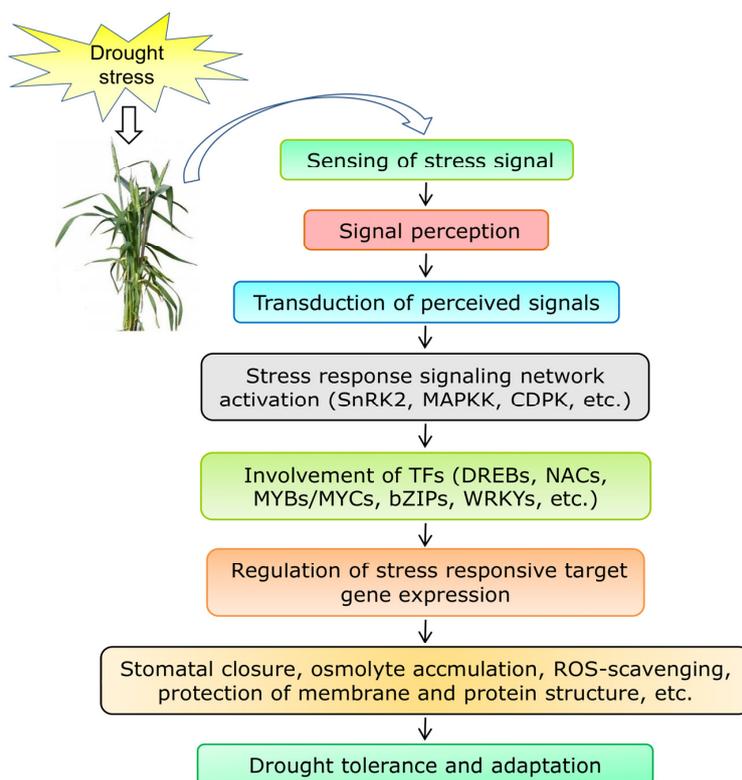


Figure 1. Steps involved in the expression of drought tolerance, starting from the perception of drought stress and transducing the signal through transcription factors for the activation of genes involved in adaptation.

A large number of QTLs have been identified for morphological and physiological traits involved in drought tolerance (Table 1). This has facilitated in developing an understanding of the genetic architecture that is responsible for providing adaptation against drought. Markers associated with these QTLs can be utilized for MAS in order to develop wheat cultivars that would be adapted to drought. The available information on the subject is being reviewed in this article.

Table 1. A summary of the morphological and physiological traits and adaptation mechanisms under drought.

S. No.	Adaptation Mechanism/Trait	Ease of Use (+/++/+++)	Reference
I. Avoidance			
1.	Leaf rolling	+++	[19]
2.	Leaf glaucousness	+++	[20,21]
3.	Shoot vigor	+++	[22,23]
4.	Transpirational cooling (cooler canopy)	++	[11,24]
5.	Stomatal conductance	+	[10]
6.	Early maturation	+++	[25]
7.	Membrane stability	+	[10,26]
8.	Green flag leaf area (stay green)	+++	[27]
9.	Root vigor and architecture	+	[28]

Table 1. Cont.

S. No.	Adaptation Mechanism/Trait	Ease of Use (+/++/+++)	Reference
II. Tolerance			
1.	Photosynthetic rate	+	[29,30]
2.	Chlorophyll content	++	[31]
3.	ABA accumulation	+	[32]
4.	Osmoprotectant accumulation	+	[33]
5.	Soluble sugar content	+	[34]
6.	Generation of reactive oxygen species (ROS)	+	[35]

+, Difficult; ++, Easy; +++, Very easy.

4. Nature of Traits/Genes Involved in Drought Tolerance

The genes involved in the synthesis of proteins, which provide drought tolerance can be broadly classified into two major groups. Genes for each group will be discussed separately.

4.1. Genes Involved in Signal Perception, Transduction, and Regulation of Transcription

The genes involved in the perception of drought and transduction of the signal include the following two major classes: (i) genes involved in the perception of the drought and immediate downstream transduction (e.g., membrane transporters, ion channels, receptor-like protein kinases, and calcium-binding proteins such as calcineurin and calmodulin); and (ii) the genes encoding transcription factors (e.g., AP2, bHLH, bZIP, C2H2, ABI3VP1, MYB, zinc fingers, MADS, NAC, and WRKY), which are involved in downstream signal transduction and eventually bind to cis-regulatory sequences of certain structural genes belonging to the second category of genes discussed in the following section.

4.2. Genes Encoding Osmoprotectants/Antioxidants and Generating Reactive Oxygen Species (ROS)

The genes involved in the protection and maintenance of cellular structure and functions have been a major target in the development of drought-tolerant crops. These genes belonging to different classes are either upregulated or downregulated and thus deal with limited water availability through a complex network. These gene systems and the regulation of their activity include the following: (i) the genes involved in cell growth that are mostly downregulated, and the genes involved in hormone synthesis (including ABA, proline metabolism, ROS-scavenging enzymes) and carbohydrate metabolism, which were activated or upregulated; (ii) genes that are expressed exclusively in roots under water-limited conditions—these genes include those related to cell expansion and encode proteins such as expansins, extensins, xyloglucans, and cellulose synthase; (iii) genes encoding protective proteins such as late embryogenesis abundant (LEA) and chaperons; and (iv) genes encoding isopentyltransferase (IPT, an enzyme that catalyzes the rate limiting step in cytokinin synthesis) leading to delayed senescence, which enables plants to maintain high photosynthetic activity during episodes of drought [36,37].

Under drought, plant development relies heavily on the timing and intensity of drought and on other environmental conditions [38,39]. Additionally, the unpredictable environmental conditions lead to poor and unreliable heritability estimates, which are crucial for genetic analyses of drought-related traits [40–42]. Responses of wheat crop to drought stress have also been reviewed recently [16,43], so the present article provides an updated and more detailed account, with an emphasis on QTL analysis.

5. Biparental Interval Mapping and Association Mapping

A large number of studies (~50) have already been conducted for the analyses of the complex genetic control of drought tolerance and related traits in hexaploid wheat and its cultivated/wild tetraploid relatives (*T. turgidum* and *T. turgidum* sp. *dicoccoides*). These studies involved interval mapping and association analyses, with most studies undertaken during the last ~5–10 years

(details are given in Tables S1–S3). In these studies, as many as ~800 QTLs/marker-trait associations (MTAs) have been reported. These QTLs and the associated markers are distributed on all the 21 wheat chromosomes, with a maximum number of QTLs/MTAs for physiological traits (429) followed by agronomic traits (318) and the root architecture related traits (23) (for a summary, see Table 2). However, only 68 QTLs (9%) of these were major QTLs (explaining $\geq 20\%$ phenotypic variation); some of these were stable QTLs (detected in $>50\%$ environments; for details of the stable QTLs, see Table 3); with other QTLs unstable (detected in $<50\%$ environments).

Table 2. A summary of studies on QTL, GWAS, and candidate gene-based association mapping for drought-related traits in wheat and its cultivated and wild tetraploid relatives (*T. turgidum* and *T. turgidum* sp. *dicoccoides*).

S. No.	Trait Class/Trait	Number of QTLs/MTAs				Range of PVE (%)
		IM	GWAS	CGAM	Total	
I. Agronomic Trait						
1.	Grain yield	84	7	-	91	02.6–39.9
2.	Thousand grain weight	60	15	-	75	00.9–45.2
3.	Test weight	13	-	-	13	03.0–10.0
4.	Grains m ⁻²	36	-	-	36	03.0–21.4
5.	Grain width	02	-	-	02	N/A
6.	Days to heading	20	10	01	31	02.4–42.4
7.	Days to flowering	03	-	-	03	07.2–11.4
8.	Days to maturity	12	04	01	17	01.7–30.1
9.	Grain-filling duration	-	02	01	03	07.1
10.	Spike density	-	03	-	03	N/A
11.	Final biomass	-	-	01	01	07.9
12.	Spikes m ⁻²	01	-	01	02	06.1–09.1
13.	Grain weight per spike	01	-	01	02	06.4–06.7
14.	Grain number per spike	02	03	01	06	04.5–12.7
15.	Flag leaf width	-	-	02	02	03.6–08.6
16.	Culm length	07	-	-	07	04.1–17.5
17.	Harvest index	14	-	01	15	01.7–22.4
18.	Spike harvest index	01	-	-	01	10.1
19.	Spike dry matter	05	-	-	05	06.6–19.1
20.	Total dry matter	03	-	-	03	09.0–11.0
II. Physiological Traits						
1.	Stem reserve mobilization	03	-	-	03	21.0–42.2
2.	Coleoptile length	68	02	-	70	00.3–65.0
3.	Canopy temperature	25	-	-	25	02.0–13.2
4.	Normalized difference vegetative index	06	02	01	09	02.0–09.0
5.	Glauconess	04	-	-	04	04.1–13.1
6.	Water soluble carbohydrates	76	06	-	82	01.1–30.0
7.	Early vigor	10	-	-	10	03.0–18.0
8.	SPAD/chlorophyll content	82	-	-	82	02.7–59.1
9.	Cell membrane stability	08	-	-	08	25.0–44.0
10.	Carbon isotope discrimination	54	-	-	54	00.8–27.4
11.	ABA content	17	-	-	17	05.1–30.0
12.	Leaf green area	-	03	02	05	04.0–04.2
13.	Leaf senescence	-	-	01	01	6.3
14.	Relative water content	01	03	-	04	06.5–17.8
15.	Osmotic adjustment	02	-	-	02	N/A
16.	Osmotic potential	10	-	-	10	02.7–08.9
17.	Photosynthetic active radiation	14	-	-	14	N/A
18.	Transpiration	14	-	-	14	N/A
19.	Leaf rolling	10	-	-	10	01.6–07.8
III. Root and Related Traits						
1.	Root length	11	-	-	11	05.0–15.6
2.	Total root biomass	02	-	-	02	09.4–10.8
3.	Root number	03	-	-	03	07.3–07.8
4.	Root dry weight	05	-	-	05	03.5–07.5
5.	Root to shoot ratio	02	-	-	02	08.0–11.0
	Total	691	60	14	763	

MTAs: marker-trait associations; IM: interval mapping; GWAS: genome wide association study; CGAM: candidate gene-based association mapping; PVE: phenotypic variation explained; N/A: not available.

5.1. Major and Stable QTLs and Their Co-Localization with Meta-QTLs (MQTLs)

A large number of major QTLs (PVE $\geq 19\%$) for agronomic and physiological traits have been reported in wheat grown under drought/water stress (Table S4). Up to ~20 environments have been used in individual studies, so that QTLs with at least ~20% PVE identified in more than 50% of the environments were considered to be stable and relatively useful. A literature search revealed only nine such stable QTLs for agronomic traits and five such stable QTLs for physiological traits (Table 3).

Two of these QTLs were co-localized with MQTLs for drought/heat stress reported earlier [44]; (for MQTLs, see later). The importance of these QTLs is discussed in the following text for different classes of traits.

Table 3. A list of major and stable QTLs with PVE ranging from 19% to 59% for agronomic and physiological traits.

S. No.	Trait/QTL	Linked Marker	Position (cM)	Env. ^a	PVE (R ²) ^b	Reference
I. Agronomic Traits						
1. Grain Yield						
(a)	<i>qGYWD.3B.2</i>	<i>Xgpw7774</i>	97.6	4/7	19.6	[45]
(b)	<i>4A</i>	<i>Xwmc420</i>	90.4	Mean/2	20.0	[46]
(c)	<i>4A-a</i>	<i>Xgwm397</i>	06.0	5/7	23.9	[24]
(d)	<i>Qyld.csdh.7AL</i>	<i>Xgwm322</i>	155.9	11/21	20.0 *	[47]
2. 1000-Grain Weight						
(a)	<i>3B</i>	<i>Xbarc101</i>	86.1	Mean/2	45.2	[48]
(b)	<i>QTgw-7D-b</i>	<i>XC29-P13</i>	12.5	10/11	21.9	[49]
3. Days to Heading						
(a)	<i>QDh-7D.b</i>	<i>XC29-P13</i>	12.5	11/11	22.7	[49]
(b)	<i>QHd.idw-2A.2</i>	<i>Xwmc177</i>	46.1	13/16	32.2	[50]
4. Days to Maturity						
(a)	<i>QDm-7D.b</i>	<i>X7D-acc/cat-10</i>	2.7	10/11	22.7	[49]
II. Physiological Traits						
1. Stem Reserve Mobilization						
(a)	<i>QSrm.ipk-2D</i>	<i>Xgwm249a</i>	142.0	2/2	42.2	[51]
(b)	<i>QSrm.ipk-5D</i>	<i>Xfbb238b</i>	19.0	2/2	37.5	[51]
(c)	<i>QSrm.ipk-7D</i>	<i>Xfbb189b</i>	338.0	2/2	21.0	[51]
2. Water Soluble Carbohydrate						
(a)	<i>QWsc-c.aww-3A</i>	<i>Xwmc0388A</i>	64.9	2/2	19.0	[52]
3. SPAD/Chlorophyll Content						
(a)	<i>Qchl.ksu-3B</i>	<i>Xbarc68</i>	67.2	2/3	59.1	[53]

^a Number of environments in which QTL was detected/number of total environments; ^b highest PVE (R²) values under drought/water stress, * with >20% higher yield per ear.

5.1.1. Biparental Interval Mapping for Agronomic Traits

Each of the above nine major and stable QTLs for drought-related agronomic traits explained ~20% to ~45% phenotypic variation (PV), which is quite substantial in view of the highly variable nature of the drought environments. Four of these QTLs were detected for grain yield, of which two QTLs were located on chromosomes 4A [24,46]. One QTL each were located on chromosomes 3B [45] and 7A [47,54]. The QTLs on chromosomes 4A and 7A also coincided with the MQTLs for drought/heat stress [44] and were mapped in genomic regions, which also harbor more than one QTL for one or more of the following traits: (i) days to heading; (ii) days to maturity; (iii) stay green habit; (iv) biomass; (v) CT; (vi) CID; (vii) coleoptile vigor; (viii) grain filling; (ix) plant height; (x) kernel number; (xi) spike density; (xii) 1000-kernal weight; (xiii) WSC; and (xiv) grain yield. These traits represent a spectrum of morphological and physiological traits contributing to seedling emergence, grain yield, and adaption to drought environments. The high PVE due to each of these QTLs and the confirmation of two of these QTLs through MQTL analyses makes them suitable for use in MAS. Therefore, the markers, *Xwmc420* and *Xgwm332* associated with these two QTLs/MQTLs may be useful for MAS aimed at breeding for drought tolerance in wheat. The marker *Xgwm332* associated with the QTL on chromosome 7A is deployed by the authors in a marker-assisted backcross-breeding (MABC) program for the improvement of yield in wheat under drought stress (for more details, see later).

As mentioned above, four of the nine major and stable QTLs for agronomic traits contributed to grain yield. The remaining five of the nine QTLs for three agronomic traits (1000-grain weight, days to heading, and days to maturity) each explained 22% to 45% PVE, although none of these QTLs was co-located with the MQTLs reported by Acuna-Gaalindo et al. [44]. As a component of grain yield, grain weight has high heritability and stability over environments and the remaining two phenological traits (early flowering and maturity) allow crops to avoid terminal water stress. Therefore, these five QTLs (associated with markers *Xbarc101*, *XC29-P13*, *Xwmc 177*, *X7D-acc/cat-10*; Table 3) may also prove useful for MAS when breeding for drought tolerance.

5.1.2. Biparental Interval Mapping for Physiological Traits

Five major and stable QTLs for three physiological traits each explaining ~20% to ~60% PV are known. One each of these QTLs are located on chromosomes 2D, 5D, and 7D (QTLs for stem reserve mobilization), 3A (QTL for WSC), and 3B (QTL for SPAD/chlorophyll content). None of these QTLs was co-located with MQTLs reported by Acuna-Gaalindo et al. [44]. Under drought, substantial stem reserves/WSC from wheat stems are remobilized to the developing grains and contribute significantly to grain development. The green leaf area in the post-anthesis period sustains carbon assimilation and contributes to grain-filling [55]. However, the leaf greenness reflects both functional (underlying photosynthetic capacity) and non-functional (cosmetic) characteristics [56], although these two characteristics are seldom phenotyped separately. Nevertheless, leaf greenness contributes significantly to grain yield, when associated with photosynthetic capacity and remobilization of stem reserve to grains [57]. Therefore, the five markers (*Xgwm294a*, *Xfbb238b*, *Xfbb189b*, *Xwmc0388a*, and *Xbarc68*) associated with QTLs for three traits (WSC, stem reserve mobilization, and chlorophyll content) may also prove useful for MAS leading to yield improvement under drought conditions.

Besides the above physiological traits, interest has also been shown in QTLs for the accumulation of the phytohormone abscisic acid (ABA), which regulates many physiological processes and contributes to the regulation of gene expression in plants under drought. Although as many as 17 QTLs for accumulation of ABA have been reported in wheat under drought environments, only half of these QTLs were major QTLs, and none was reported as stable ([58,59]; for details, see Table S4). A QTL associated with ABA level flanked by SSR markers *Xpsr575* and *Xpsr426* on chromosome arm 5AL [58] was associated with dehydrin genes (*Dhn1/Dhn2*) and showed a direct association between ABA accumulation and drought tolerance [60]. The genes for ABA signaling were reported in the genomic region [61] that harbors an important QTL on 7A, which controls several traits including the grain yield per spike ([47,54]; for details, see Section 5.1.1 above). In response to drought, 11 major QTLs for ABA content on four different chromosomes (3A, 4A, 5A, and 7B) were also reported [59]. Genes underlying these QTLs may be investigated using functional genomics tools to further elucidate the role of ABA in drought stress response.

5.2. Meta-QTLs and Their Associated Candidate Genes

A meta-QTL analysis was also conducted, which involved 502 QTLs for physiological and agronomic traits reported in 30 studies conducted under conditions of drought [44]. As many as 19 MQTLs for drought tolerance spread over 13 chromosomes were reported. Each MQTL represented 2–8 individual QTLs and the 19 MQTLs represented individual QTLs for a total of 17 different agronomic and physiological traits (Table 4). Each MQTL had much narrower confidence interval (average 5.8 cM) than the confidence intervals (average 21.6 cM) of individual QTLs, suggesting more precision in the mapping of MQTLs. Four of the 19 MQTLs (MQTL2, MQTL11, MQTL29, and MQTL61) each represented six to seven individual QTLs for agronomic and physiological traits. The agronomic traits included coleoptile vigor, kernel number, grain yield, biomass, HI, plant height, spike density, 1000-grain weight, heading/anthesis, and maturity, while the physiological traits included CID, stay green, WSC, grain filling, water status, and photosynthesis. All the above agronomic and physiological traits are important for breeding for drought tolerance; therefore, the markers (*Xwmc11*, *Xwmc296*, *Xgwm314* and *Xgwm400*) associated with these four MQTLs may prove useful for MAS when breeding for drought tolerance.

Candidate genes underlying the five MQTLs (MQTL2, MQTL18, MQTL42, MQTL51, and MQTL66) were also reported by Acuna-Galindo et al. [44]. The candidate genes for these MQTLs are listed in Table 4 and are mainly involved in antioxidative activity, stress signaling, and protein storage; some of these candidate genes also seem to be involved in regulation of vesicular traffic. Thus, candidate gene-based association mapping involving the above genes should allow for the identification of causal SNPs for use in MAS for wheat breeding for drought tolerance.

Table 4. A summary of MQTLs for drought tolerance in wheat and their associated candidate genes (based on Acuna-Gaalindo et al. [44]).

MQTL	Chr.	Linked Marker	Traits for Individual QTL Representing MQTL	Co-Localized Candidate Gene ID ^a	Predicted Function
MQTL2	1A	<i>Xwmc11</i>	CID, CL, KN, SG, WSC, YLD	1. Ta.11441.3 2. Ta.24298.1 3. Ta.1257.2	1. ADP-ribosylation factor1 2. Prolamin, 2, 26 kDa globulin, Alpha globulin 3. Prolamin subfamily 2
MQTL3	1A	<i>Xwmc51</i>	PS, WSC	-	-
MQTL11	2A	<i>Xwmc296</i>	Bio, CID, CL, GF, HI, WSC, WS	-	-
MQTL14	2B	<i>Xwmc489</i>	HI, PH, KN, SG	-	-
MQTL16	2B	<i>Xbarc7</i>	BIO, CL, HI, WS	-	-
MQTL18	2B	<i>Xgwm47</i>	PH, SG, WSC, YLD	1. Ta.8144.1 2. Ta.9253.1	1. Gamma-SNAP 2. SIT4 phosphatase
MQTL21	2D	<i>Xwmc601</i>	CID, CL, WSC	-	-
MQTL22	2D	<i>Xgwm539</i>	CID, SG, TKW, YLD	-	-
MQTL23	3A	<i>Xwmc11</i>	TKW, WS	-	-
MQTL29	3D	<i>Xgwm314</i>	CL, PH, PS, SD, TKW, YLD	-	-
MQTL42	5B	<i>Xwmc73</i>	PH, YLD	1. Ta.9194.1	1. L-ascorbate:Na symporter
MQTL46	5D	<i>Xgwm358</i>	CL, PS, WSC	-	-
MQTL50	6A	<i>Xgwm427</i>	CID, TKW	-	-
MQTL51	6B	<i>Xgwm508</i>	HI, KN, WS, YLD	1. Ta.13551.1 2. Ta.5227.2	1. SurE 2. S-adenosylmethionine synthetase 1
MQTL53	6B	<i>Xbrac198</i>	CL, WSC	-	-
MQTL56	6D	<i>Xwmc773</i>	CID, YLD	-	-
MQTL61	7B	<i>Xgwm400</i>	HD, BIO, CID, HI, MD, WS, YLD	-	-
MQTL64	7D	<i>Xcfd66</i>	PS, WSC	-	-
MQTL66	7D	<i>Xwmc659</i>	PS	1. Ta.1055.1	1. Catalase isozyme A

^a Wheat HarvEST Unigene ID; BIO: biomass; CID: carbon isotope discrimination; CL: coleoptile vigor; GF: grain-filling; HD: heading/anthesis; PH: plant height; HI: harvest index; KN: kernel number; MD: maturity; PS: photosynthesis; SG: stay-green; SD: spike density; TKW: thousand kernel weight; TW: test weight; WS: water status; WSC: water-soluble carbohydrates; YLD: yield.

5.3. Biparental Interval Mapping and Epistatic QTLs

Epistasis has been shown to contribute substantially to the genetic variation for a number of complex traits (e.g., adaptation to drought, heat, and salinity) in crops including wheat and other cereals [62–64]. Therefore, the identification of epistatic QTLs is essential for the development of efficient marker-assisted selection (MAS) schemes for complex traits such as drought tolerance, aimed at improving breeding efficiency [65]. However, only a few QTL studies have been conducted, which included detection of QTL × QTL interactions for adaptation to drought/water stress conditions.

Using studies conducted in the field or green houses/PVC pipes, at least 108 QTL × QTL interactions were reported for three agronomic and four physiological traits (Table 5 and Table S5). Many of these epistatic interactions also involved main-effect QTLs. It is thus clear that both the main-effect QTLs and the epistatic QTLs (with or without main effects) are known for drought tolerance. Higher order interactions such as QTL × QTL × QTL may also contribute to the total genetic variation, but the study of these higher-order interactions still remains a challenge due to high computational demand.

The PVE due to each pair of the interacting epistatic QTLs was very low (0.27% to 8.26%), suggesting that the epistatic interactions do not play a major role and that perhaps it is the main-effect QTLs that provide tolerance to drought. A word of caution here seems to be necessary because, in the majority of QTL studies, the population size and the methodology used perhaps would not allow detection of all epistatic interactions.

Table 5. A summary of pairs of QTLs for different traits involved in epistatic interactions under drought in wheat.

S. No.	Trait Class/Trait	QTL × QTL Pairs	PVE (%) Range	Reference
I. Agronomic Trait				
1.	Grain yield	04	0.51	[45,50,66]
2.	Thousand grain weight	24	0.59–8.26	[45,66–68]
3.	Days to flowering	12	0.30–1.40	[45,67,69]
II. Physiological Traits				
1.	Coleoptile length	04	0.50–2.70	[70]
2.	Water soluble carbohydrates	24	0.84–5.61	[68]
3.	Carbon isotope discrimination	02	N/A	[66]
4.	SPAD/chlorophyll content	38	1.08–3.29	[71]
Total		108	0.30–8.26	

Five of the above 108 QTL × QTL interactions each had more than 5% PVE and thus may be considered for use in MAS, while breeding for drought tolerance (Table 6). These included four epistatic interactions for 1000-grain weight and one epistatic interaction for WSC that were reported using populations grown under field studies. Since none of these interactions involved main-effect QTLs, these may be important for exploitation of epistatic genetic variation for 1000-grain weight and/or WSC in a breeding programme.

Table 6. Important epistatic interaction (QTL × QTL) with PVE ≥ 5% reported in wheat under drought/water stress (Yang et al. [68]).

Trait	QTL _i QTL/Chromosome	Associated Marker; Position (cM)	QTL _j QTL/Chromosome	Associated Marker; Position (cM)	PVE
TGW	1. <i>QTgwg.cgb-1B</i>	P3622-280; 0	<i>QTgwg.cgb-5A</i>	<i>Xwmc524</i> ; 0	5.16
	2. <i>QTgwg.cgb-4A.2</i>	CWM145; 9	<i>QTgwg.cgb-4A.3</i>	<i>XP4232-260</i> ; 3	8.26
	3. <i>QTgwg.cgb-6A.2</i>	Xgwm334; 0	<i>QTgwg.cgb-6A.3</i>	<i>XP3474-260</i> ; 2	5.79
	4. <i>QTgwm.cgb-2B.1</i>	P6411-216; 0	<i>QTgwm.cgb-7B.4</i>	<i>Xwmc276</i> ; 1	6.61
WSC	1. <i>QSwscg.cgb-2B</i>	WMC441; 5	<i>QSwscg.cgb-6B</i>	<i>Xwmc182</i> ; 0	5.61

TGW: 1000-grain weight; WSC: water-soluble carbohydrates.

5.4. MTAs Identified through GWAS

At least four genome-wide association mapping studies (GWAS) involving 108–262 genotypes have been conducted in wheat under drought [72–75], and 60 marker-trait associations (MTAs) were detected for several agronomic and physiological traits. The markers used included genome-wide SSR, SNP, and DArT markers (Table S2). Due to a lack of shared markers among the above studies on GWAS and those on interval mapping/meta-QTL analyses (discussed above), it was not possible to relate the MTAs identified through GWAS with those mapped through interval mapping. None of these four studies applied Bonferroni correction for the identification of true MTAs, although two of these studies [72,75] applied FDR (false detection rate) criteria, which is relatively less stringent, so the above 60 MTAs may also include false positives. In view of this, it would be desirable to validate these important MTAs through QTL interval mapping using bi-parental populations before considering these MTAs for MAS.

Out of the above four studies involving GWAS, the PVE due to MTAs was reported in only one of them. In this study, PVE due to MTAs for 1000-grain weight, coleoptile length, and relative water content ranged from 6.5% to 17.8% ([73]; Table S2). The highest PVE was due to an SSR locus *Xgwm312* associated with relative water content; this association could be exploited in breeding for drought tolerance. In another *in silico* study, six candidate genes associated with MTAs were identified ([75]; for details on SNP, chromosome, and traits involved in MTAs and the corresponding genes, see Table 7). These genes are involved in one or more of the following processes: chondroitin sulfate biosynthesis

and glycan structures biosynthesis 1, pathway protein ubiquitination and in-protein modification, maintenance of immune self-tolerance, synthesis of glycoprotein and glycosphingolipid sugar chain, protein binding, etc. These six candidate genes should be the subject of future studies.

Besides GWAS, candidate gene-based association mapping involving the following five genes that are involved in drought tolerance in wheat have also been carried out [76]: (i) *DREB1A* (dehydration responsive element binding); (ii) *1-FEH-A* and *1-FEH-B*, each for fructan-exohydrolase; and (iii) *ERA1-B* and *ERA1-D*, each for enhanced response to abscisic acid (ABA). Sequence variation of the genes was examined in a spring wheat association mapping panel consisting of 126 genotypes. For each individual gene, one (*1-FEH-B*) to four (*ERA1-D*) causal SNPs were detected. Details are as follows: (i) two SNPs for *DREB1A* were associated, one each with days to heading and final biomass; (ii) one SNP for *1-FEH-B* was associated with days to maturity; (iii) in the case of *1-FEH-A*, three SNPs were associated with three traits (grain number per spike, NDVI, and green leaf area, respectively), and another SNP was associated with a solitary trait (green leaf area); (iv) in the case of *ERA1-B*, two SNPs were associated, one each with grain filling duration and spike number per m²; and (v) out of the four SNPs that were detected in *ERA1-D*, one SNP was associated with grain weight per spike and flag leaf width; the remaining three SNPs were associated, one each with flag leaf width, harvest index, and leaf senescence. These SNPs may be exploited in MAS, after due validation.

Table 7. Description of MTAs associated with yield and its related traits under drought in wheat and their possible candidate genes (more details are given in Ain et al. [75]).

S. No.	Marker Name	Chr.	Trait	Candidate Gene
1.	Tdurum_contig80278_250	1AL	GY	Galactosylgalactosylxylosylprotein 3-beta-Glucuronosyl transferase 1
2.	Excalibur_c8052_541	1BS	DTH	e3 ubiquitin-protein ligase herc2
3.	RAC875_rep_c77617_1454	2AL	TGW	Serine threonine-protein phosphatase 6 Regulatory subunit 3-like isoform x1
4.	BS00022025_51	3BL	TGW	Glycosyltransferase-like protein
5.	RAC875_c23144_1560	4BL	GY	Upf0202 protein at1g10490-like
6.	tplb0024a09_742	7DS	GY	Rna polymerase ii transcription partial

Chr.: chromosome; GY: grain yield; DTH: days to heading; TGW: 1000-grain weight.

5.5. Genes Encoding Transcription Factors (TFs) and Involved in a Two-Component System (TCS)

A number of genes encoding TFs and those involved in TCS are relevant to drought tolerance [18,77]. These genes were also assigned to specific chromosomes through sequence comparison. Interestingly, 45 TF/TCS genes were mapped on 16 wheat chromosomes/arms that were already known to harbor 56 major QTLs for the following 13 traits under drought: (i) cell membrane stability; (ii) SPAD/chlorophyll content; (iii) days to heading; (iv) days to maturity; (v) stem reserve mobilization; (vi) WSC; (vii) ABA accumulation; (viii) grain yield; (ix) 1000-grain weight; (x) coleoptile length; (xi) CID; (xii) harvest index; and (xiii) grains m⁻² (Table S6). The genes for TF/TCS may be mapped using suitable mapping populations using markers associated with the above QTLs to help determine the coincidence of the TF/TCS genes and QTLs, if any.

6. Molecular Marker-Assisted Breeding

6.1. Marker-Assisted Backcrossing (MABC)

During the last decade, several important and major QTLs for drought-mediated grain yield and its components have become available in wheat (for details, see Section 5). However, MABC has only been rarely attempted for the improvement of drought tolerance in wheat on a large scale. For example, under the Generation Challenge Programme funded by CIMMYT, Mexico, and the National Initiative on Climate Resilient Agriculture Project supported by ICAR, India, efforts were made to introgress

QTLs for several drought-related traits (canopy temperature, chlorophyll content, stay green habit, NDVI values, days to anthesis, grain yield, and its related traits) into two elite Indian wheat cultivars (HD2733 and GW322) through MABC [78] (for details of QTLs, see Kirigwi et al. [46], Pinto et al. [24], Kumar et al. [53], and Kadam et al. [69]). After foreground and background selection (90% recurrent parent), the BC₁F₂- and BC₂F₂-containing QTLs for drought-related traits and the 90% genome of the recurrent parent genotypes were advanced for seed multiplication. These progenies (BC₁F₅/BC₂F₄) are now being evaluated for their field performance under rain-fed environments. It is hoped that some of these progenies will certainly out-yield their respective recipient parents under water stress environments, leading to the development of drought-tolerant cultivars (Neelu Jain, IARI, New Delhi, India; personal communication).

In our own laboratory (at Meerut, India), we are undertaking MABC using an SSR marker (*Xwmc273*) that is associated with a major QTL (*Qyd.csdh.7AL*) for grain weight per ear (contributing to a 20% hike in grain yield) under stressed environments [47,54]. Using MABC, desirable allele from the donor cultivar SQ1 were successfully introgressed into four drought sensitive Indian bread wheat cultivars (HUW234, HUW468, K307, and DBW17; our unpublished data). The MABC-derived progenies in the BC₂F₅ generation were tested for seven agronomic traits (including grain yield) and two physiological traits (i.e., chlorophyll content and canopy temperature depression). The preliminary analyses suggested that at least seven progenies in the background of HUW234 and one progeny each in the backgrounds of HUW468 and K307 when tested at two different locations (one progeny was common) exhibited a yield advantage of 21.6% to 59.4% over the respective recipient parent under drought conditions. The improvement in grain yield of the progenies was associated with an improvement in several other agronomic and physiological traits. For example, each of the high yielding BC₂F₅ progenies in the background of HUW234 was also significantly superior in several of the following traits: grain number per ear, grain weight per ear, thousand grain weight, tiller number per meter, biological yield per plot, and canopy temperature depression. Similarly, one high yielding progeny each in the backgrounds of HUW468 and K307 was also superior in the following different traits: grain weight per era, thousand grain weight, tiller number per meter, and canopy temperature depression. Interestingly, all high yielding progenies exhibited superiority for grain weight per ear that is controlled by the introgressed major QTL (*Qyd.csdh.7AL*) [47,54], suggesting that the QTL effect is expressed in the different genetic backgrounds as well as at different locations in some of the improved progenies. Currently, the MABC-derived progenies are being tested at three locations under irrigated and rain-fed environments to assess their potential for release as cultivars.

In another recent study, desirable alleles from some QTLs from wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) were also introgressed into durum and bread wheat cultivars [79]. Two QTLs (one each on 1BL and 2BS) were validated in the background of durum wheat and one QTL (7AS) in the background of bread wheat. Improved grain yield and biomass under drought was obtained due to one QTL on 7AS in bread wheat, and another QTL (2BS) in durum wheat. Therefore, besides the exploitation of wheat gene pool, there is also a need to explore the wild relatives of wheat for the identification of QTLs for drought tolerance.

6.2. Marker-Assisted Recurrent Selection (MARS)

MARS for the improvement of WUE in wheat was attempted in an Indo-Australian project. The project involved partners from the ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR), Karnal, PAU Ludhiana, and the ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi and Australia. The Generation Challenge Programme (GCP) of CGIAR system also launched an initiative to improve heat/drought tolerance in wheat through the MARS approach. This program involved ICAR-IARI, New Delhi, India, Chinese Academy of Agricultural Sciences (CAAS), China, and partners from Australia [80]. Under the GCP programme, at ICAR-IARI, New Delhi, attempts were also made to combine QTLs for stress adaptive traits, such as early vigor, SPAD values at vegetative and reproductive stages, NDVI, chlorophyll fluorescence, and flag leaf

area, following MARS [78]. Progenies segregating for QTLs for the above traits in the two F₄ base populations were subjected to foreground selection to identify those progenies, which carried desirable combinations of QTLs and excelled in yield performance during multi-location trials. These selected progenies were subjected to inter-family intermatings in the F₅ generation. The progenies derived from these intermatings were superior in performance to their parents as well as the check cultivar HD3043; the promising lines are being subjected to station yield trials. This program is an example of the success of MARS in wheat improvement for drought tolerance [78].

7. Future Perspectives

The methods for genotyping and phenotyping and the statistical tools for QTL analysis have been undergoing major changes in recent years, leading to significant improvements in precision and the speed of conducting QTL analysis (both interval mapping and GWAS). Future possibilities involving some of these advances in QTL analysis will be briefly described in this section.

7.1. High Throughput Phenotyping

The slow progress in high-throughput field phenotyping (HTFP) has become a 'bottle-neck' in breeding programmes for drought tolerance. A variety of non-invasive imaging techniques have been used to develop different platforms for high-throughput automated and integrated phenotyping of large plant populations with high resolution and high precision [81–91]. These techniques include fluorescence imaging, thermal infrared imaging, visible light imaging, imaging spectroscopy, and multispectral imaging, among others. The ground-based and unmanned aerial HTFP platforms that were developed for real-world phenotyping of above-ground traits include the following: (i) phenomobiles; (ii) pheno-fields; (iii) breedvision; (iv) phenocart; (v) pheno-towers; (vi) blimps; and (vii) infrared imagery (IR radiation sensor mounted on a light aircraft) [92–95]. However, the cost of HTFP platforms is rather high (cost \$100,000 [96]), although recently, cheaper platforms such as "Phenocart" (cost \$12,000) have also become available [97]. These platforms will be increasingly used in future for the phenotyping of traits that are relevant to drought tolerance [57].

The progress in the area of the HTFP for root system architecture (RSA) under field conditions is rather slow. Some non-destructive methodologies, including ultrasound, magnetic resonance imaging, computed tomography (CT), and X-rays have been developed for phenotyping of RSA in soil systems (for details, see Selvaraj et al. [98]). These high-resolution phenotyping approaches have low-throughput (reviewed in Mooney et al. [99]) and their potential use in HTFP in wheat breeding programmes for drought tolerance remains to be tested. The available HTFP platforms provide an integrated complex data (i.e., big data), so that suitable statistical data analyses pipelines are also needed [100,101]. Major efforts are needed for the development of cheaper and user-friendly platforms for routine use in breeding programmes of average size for the real-world phenotyping of shoot and root traits.

7.2. High Throughput Genotyping

In recent years, SNP chips and GBS have been increasingly used for high throughput SNP genotyping. This facilitated identification of markers, closely associated to the QTLs for different traits. However, SNP genotyping in wheat has been seldom used in experiments involving genetic analyses of drought tolerance. In future, this will also facilitate the discovery of candidate genes underlying drought QTLs. A recent workshop on "Wheat Genomic Resources in a Post Reference Sequence Era" organized by the "Wheat Initiative" during 6–7 July 2016 at Cambridge (UK) emphasized that the cost of SNP genotyping needs to be brought down before it is within the reach of an average breeding programmes [102].

7.3. Cloning of Genes Underlying QTLs for Drought Tolerance Related Traits

Genes underlying QTLs for several different traits have been cloned in maize, rice, and sorghum (for details, see review by Salvi and Tuberosa [15]). However, due to the large size of the wheat genome and ~80% repetitive DNA, map-based cloning of genes underlying the QTLs for different traits has been undertaken only sparingly. Examples of successful cloning of genes for QTLs in wheat include the following: (i) root-specific boron transporter genes underlying the two major-effect QTLs (*Bo1* and *Bo4*) for boron tolerance in wheat [103]; and (ii) pore-forming toxin-like (PFT) gene underlying *Fhb1* QTL for fusarium head blight tolerance [104]. With the availability of several major and stable QTLs for drought-related traits in wheat, efforts are needed for the cloning of the genes underlying such QTLs. Characterization of genes underlying the QTLs for drought tolerance would help (i) in better understanding of the molecular mechanism of drought tolerance, and (ii) in the development of gene-based functional markers for direct use in breeding programmes aimed at the improvement of drought tolerance.

7.4. Genetical Genomics and eQTLs

The genes showing variation in their expression could be studied through expression quantitative trait loci (eQTL) mapping and the genetical genomics (large scale analyses of genetic regulation of entire transcriptomes) could be used to elucidate the biochemical pathways of interacting genes on the basis of variations in transcript levels. The eQTLs could also help in the identification of genes underlying a QTL for a phenotypic trait. Although eQTL analyses under drought has been carried in several plant species [105], only one study is available in durum wheat; this study involved mapping of an eQTL for *HEL* (high level expression gene), using a mapping population derived from two genotypes differing for WUE [106]. Therefore, more studies are required in the area of genetical genomics and eQTL analyses to help elucidate the regulation of individual gene expression and the biochemical pathways of the interacting genes in wheat under drought.

7.5. EpiQTL for Drought Tolerance

In any individual crop, the inheritance of almost all agronomic traits that have been examined so far have a certain component of epigenetics control (either DNA methylation or histone modification, or both). Therefore, it is natural to expect epigenetic control of the expression of genes involved in response to drought as well. In this connection, preliminary information regarding hypermethylation and hypomethylation in response to drought (which is also transgenerational in nature in some cases) has been reported in some plants systems including rice [107–109]. Even epiQTLs involving DNA methylation have been reported for flowering time and primary root length in *Arabidopsis* [110]. However, the role of epigenetic control of drought responsive traits in wheat has yet to be examined, and epiQTLs have yet to be identified; epiRILs will have to be developed for this purpose. The genes underlying the epiQTLs may be discovered using wheat genome resources available at <http://plants.ensembl.org/index.html>, and an understanding of the role of epigenetics in quantitative trait variation under drought may be developed.

7.6. Alien Genetic Variation for Drought Tolerance

Alien species related to wheat are widely known as a reservoir of novel genes/traits for biotic and abiotic stresses for wheat improvement. However, only a few attempts have been made towards the discovery and exploitation of alien QTLs for drought-related traits for wheat breeding. Therefore, a major effort is needed to exploit this untapped resource of alien species for improving drought tolerance in wheat [111]. The sources of alien genetic variation may include not only durum and emmer-based synthetic hexaploid wheats, but also other alien species belonging to the genera *Secale*, *Aegilops*, and *Agropyron*. In a study involving a number of synthetic wheats, a significant correlation of drought tolerance index with root biomass, length of the longest root, stomatal conductance,

and production of roots with small diameter was noticed, suggesting the importance of synthetic wheats in breeding for drought tolerance [112]. A classic example of the use of alien species is the presence of 1BL.1RS translocation in many important wheat varieties. The rye 1RS arm carries genes for adaptation to abiotic stresses, including a robust drought-tolerant root system, besides genes for resistance to several diseases [113]. Using disomic addition lines of *Agropyron elongatum* in wheat cv. Chinese Spring, several chromosomes of *A. elongatum* were shown to carry genes/QTLs for following traits contributing to drought tolerance: yield under stress, grains per plant, grains per spike, seed weight, relative water content (RWC), and leaf water potential (LWP) [114]. These examples illustrate the need for screening alien species for the variability for drought tolerance.

7.7. Physiological Trait Based Breeding

Breeding for drought tolerance has been largely based on selection for grain yield. As an alternative to empirical breeding, physiological breeding has been suggested as a possible route to break the yield barriers [115]. Physiological breeding often encompasses a larger range of traits including genetically complex physiological traits (e.g., osmotic adjustment, accumulation and remobilization of stem reserves, superior photosynthesis, heat- and desiccation-tolerant enzymes, canopy temperature, and root system architecture) as well as phenomic and genomic information [115–118]. According to Reynolds and Langridge [115], the following key steps are involved in physiological breeding: (i) designing a plant type with improved adaptation; (ii) the identification of genetic resources encompassing new and/or complementary allelic variation (for crossing); (iii) developing and implementing phenotyping protocols and experimental treatments to maximize resolution of physiological trait expression (to select parents); (iv) genetic dissection of traits and the development of gene-based selection approaches; (v) strategic hybridization among properly characterized genotypes for physiological traits to achieve cumulative gene action for yield, combined with the application of high throughput phenotyping and genotyping to select progeny; (vi) the analysis of trait/allele combinations that achieve environmentally robust genetic gains based on multi-location trial data (to design new crosses); (vii) informatics services underpinning the iterative refinement of breeding strategies across all steps. Considering the importance of physiological breeding, it has become central to the newly launched International Wheat Yield Partnership [119], which is aimed at raising a wheat yield potential that is closer to its biological limit as well as HeDWIC initiatives of the CGIAR that aim to adapt crops to climate change for global food security.

8. Conclusions

As discussed in this article, some major QTLs/MQTLs are now known for drought tolerance related traits. Therefore, it should now be possible to design programs for wheat breeding based on MARS for rapid advances in breeding for drought tolerance in wheat. Epistatic QTLs and epiQTLs will also be discovered in future to be used for molecular breeding in wheat. There is also a need to explore genomic selection (GS) for drought tolerance, which has already been used for the improvement of other traits, particularly disease resistance in wheat [120–122].

Supplementary Materials: The following supplementary material are available online at www.mdpi.com/2073-4395/7/1/5/S1. Table S1: List of QTLs for different traits detected in wheat and its two related tetraploid species (*T. turgidum* and *T. turgidum* ssp. *dicoccoides*) under drought; Table S2: List of MTAs for different traits detected using GWAS in wheat under drought; Table S3: List of SNP markers identified for drought tolerance related traits based on candidate gene-based association mapping in wheat; Table S4: Major QTLs for 13 different agronomic and physiological traits reported in wheat under drought/water stress; Table S5: QTLs for different traits involved in epistatic interactions in wheat under drought; Table S6: TF/TCS genes that were mapped on 16 wheat chromosomes/arms known to harbor major QTLs for drought tolerance in wheat.

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