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Master's thesis

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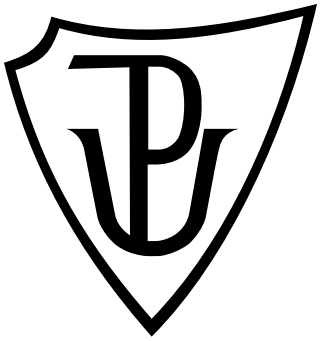

High-density mapping of agronomically important traits using a reference genome sequence of wheat

Master's thesis

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- Genomická selekce linií
- Integrace dostupných map
- Ukotvení do referenční sekvence
- Design nových markerů
- Vysokohustotní mapování
- Zpracování literární rešerše, vyhodnocení výsledků a jejich interpretace

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- MANTOVANI, P., M. MACCAFERRI, M. C. SANGUINETI a kol. An integrated DArT-SSR linkage map of durum wheat. Molecular Breeding. 2008, 22(4), 629-648. International Wheat Genome Sequencing Consortium (IWGSC). Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018, 361(6403).
- DUBCOVSKY, J., M. C. LUO, G. Y. ZHONG a kol. Genetic map of diploid wheat, Triticum monococcum L., and its comparison with maps of Hordeum vulgare L. Genetics. 1996, 143(2), 983999.
- DOROSHKOV, A. V., T. A. PSHENICHNIKOVA a D. A. AFONNIKOV. Morphological characterization and inheritance of leaf hairiness in wheat (Triticum aestivum L.) as analyzed by computer-aided phenotyping. Russian Journal of Genetics. 2011, 47(6), 739-743.

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Abstract:

Common wheat is the leading cereal crops worldwide and plays a major role in sustaining food security. It is an allohexaploid species with a large genome, which complicates wheat genomic studies. Diploid wheat ancestors are employed to ease some of the difficulties. Diploid einkorn wheat *Triticum monococcum* is closely related to the A genome of common wheat. Einkorn's smaller diploid genome, the availability of both cultivated and wild forms with broad gene pool, and a high level of gene conservation and collinearity with other *Triticum* species make it an attractive model for mapping of quantitative trait loci (QTLs). A linkage map of *T. monococcum* was constructed using 81 F_8 recombinant inbred lines derived from a cross of cultivated *T. monococcum* ssp. *monococcum* 'DV92' and wild *T. monococcum* ssp. *boeoticum* 'G3116'. The linkage map contains 676 molecular markers assigned to seven linkage groups and covers 1033 cM with one marker per 1.53 cM on average. A total of 129 QTLs for 17 quantitative traits (including plant height, the number of tillers and their pattern, leaf pubescence, ear emergence time, spike traits, and grain traits) were mapped. Leaf pubescence provides a protection against both biotic and abiotic stress factors. Two highly significant QTLs for leaf pubescence were mapped on chromosomes $3A^m$ and $5A^m$ and the markers associated with the QTLs were aligned against the physical map of common wheat within a 20 Mbp interval on 3B and a 1.2 Mbp interval on 5A, respectively. Both QTLs were verified on verification mapping populations. This makes it an ideal basis for identification of genes underlying the trait.

Keywords:	Wheat, *Triticum monococcum*, Quantitative trait loci, Linkage mapping
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Abstrakt:

Pšenice setá je jednou z nejdůležitějších obilnin a hraje významnou roli v celosvětovém zajišťování potravin. Jedná se o allohexaploidní druh s velkým genomem, což ztěžuje jeho výzkum. Diploidní předci pšenice se proto využívají pro zjednodušení některých aspektů výzkumu. Diploidní pšenice jednozrnka *Triticum monococcum* je blízce příbuzná s donorem genomu A pšenice seté. Menší diploidní genom, existence kultivovaných i divokých forem, která je zdrojem velkého počtu alel genů, a vysoká mezidruhová konzervovanost a kolinearita genů z jednozrnky činí atraktivní model pro mapování lokusů kvantitativních znaků (QTL). Vazebná mapa *T. monococcum* byla sestavena s použitím 81 F_8 rekombinantních inbredních linií odvozených z křížení kultivované *T. monococcum* ssp. *monococcum* 'DV92' a divoké *T. monococcum* ssp. *boeoticum* 'G3116'. Vazebná mapa obsahuje 676 molekulárních markerů řazených do sedmi vazebných skupin a pokrývá 1033 cM s v průměru jedním markerem na 1,53 cM. Celkem bylo zamapováno 129 QTL pro 17 kvantitativních znaků (včetně výšky rostliny, počtu odnoží a typu odnožování, chlupatosti listů, doby metání a znaků spojených s klasem a zrnem). Chlupatost listů rostlině poskytuje ochranu před biotickým i abiotickým stresem. Dva vysoce signifikantní QTL pro chlupatost listů byly zamapovány na chromozomech $3A^m$ a $5A^m$ a markery asociované s těmito QTL byly přiřazeny na fyzickou mapu pšenice seté ve 20 Mbp intervalu na 3B a 1,2 Mbp intervalu na 5A. Oba QTL byly verifikovány na verifikačních mapovacích populacích. Toto činí předloženou analýzu ideálním základem pro identifikaci genů ovlivňujících mapované znaky.

Klíčová slova: pšenice, *Triticum monococcum*, lokusy kvantitativních znaků, vazebné mapování

Počet stran: 101

Počet příloh: 7

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Declaration

I declare that this thesis has been composed by myself and is entirely my own work, except where stated otherwise by reference or acknowledgment, and that used sources are listed in the References section.

The work was done under the guidance of Mgr. Miroslav Valárik, Ph.D. at the Institute of Experimental Botany of the AS CR, v.v.i. in Olomouc, the Czech Republic.

Olomouc, May 22, 2020 Adam Lampar

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Abbreviations

AFLP	Amplified fragment length polymorphism
AM	Association mapping
BAC	Bacterial artificial chromosome
BC	Backcross
bp	Base pairs
C	C-value; 1C represents the amount of DNA in a haploid chromosome set
CAPS	Cleaved amplified polymorphic sequence
CIM	Composite interval mapping
cM	centiMorgan
DArT	Diversity Arrays Technology; diversityarrays.com
DH	Doubled haploid
EST	Expressed sequence tag
F primer	Forward primer
F_n	The *n*th filial generation
Gbp	Giga base pairs
ICIM	Inclusive composite interval mapping
IM	Interval mapping
IRAP	Inter-retrotransposon amplified polymorphism
IWGSC	International Wheat Genome Sequencing Consortium; wheatgenome.org
JLAM	Joint linkage association mapping
LD	Linkage disequilibrium
LG	Linkage group
LOD	Logarithm of the odds ratio
LP	Length polymorphism
M	Morgan
MAGIC	Multiparent advanced generation inter-cross
Mbp	Mega base pairs
MIM	Multiple interval mapping
n	Haploid number
NAM	Nested association mapping
NGS	Next-generation sequencing
NIL	Nearly isogenic line
PA	Presence-absence polymorphism
PCR	Polymerase chain reaction
PEV	Percentage of explained variance
QTL	A quantitative trait locus
QTLs	Quantitative trait loci
R primer	Reverse primer
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
SIM	Simple interval mapping
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
STS	Sequence-tagged site
x	Monoploid number

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1 Introduction

Wheat is one of the most widely grown crops in the world and is critical for human nutrition. With the rapidly growing world population and changing climate, the demand for wheat is increasing and high-yielding and stress-resistant cultivars will be required more than ever before.

Diploid einkorn wheat, *Triticum monococcum* L., is closely related to *T. urartu*, the donor of the A genome of common wheat, and was among the first cereals domesticated by Neolithic farmers more than 10,000 years ago in the Fertile Crescent. However, its popularity started to decrease during the Bronze Age when higher-yielding free-threshing tetraploid and hexaploid wheats became cultivated more extensively and einkorn grew in its natural environment without selection for thousands of years. Therefore, einkorn's diploid nature, a high level of gene collinearity with other *Triticum* species, and a large number of phenotypic variations make it a useful model for wheat genomic studies and a valuable source of genes for an enrichment of common wheat gene pool.

To assess the genetic architecture of agronomically important traits, a recombinant inbred line population of cultivated and wild einkorn was employed to map loci underlying agronomically important traits.

2 Aims and objectives

This thesis builds on previous studies (Dubcovsky et al., 1996; Vanžurová, 2013; Lampar, 2018) and aims to finish mapping of QTLs for agronomically important traits in *T. monococcum* L. using a linkage map developed from a cross of cultivated *T. monococcum* ssp. *monococcum* 'DV92' and wild *T. monococcum* ssp. *boeoticum* 'G3116'. It especially aims to map QTLs for grain traits, which have not been previously assessed by Vanžurová (2013), and verify and fine map QTLs for leaf pubescence.

The objectives were set as follows:

1) Phenotyping of grain traits
2) Linkage map construction
3) QTL analysis
4) Marker saturation of two chromosomal regions with the QTLs for leaf pubescence
5) Literature review, data evaluation and interpretation of results

3 Literature background

3.1 Wheat

Global food production will have to be increased by about 60% by 2050 to satisfy the world's growing population (Alexandratos & Bruinsma, 2012). An additional 593 million hectares of agricultural land would be needed to achieve this goal. It would come at a heavy price of deforestation causing massive habitat loss. Additionally, it would release carbon stored in the soils and further enhance the climate change (Searchinger et al., 2019). Plenty of other measures will have to be taken to minimize such undesirable events. One of the solutions could be improved high-yielding crop breeding.

Cereals, such as wheat, maize, and rice, provide more than 50% of calories and proteins for the world's population. In 2019, global cereal production reached 2.72 billion tons, out of which global wheat production accounted for 762 million tons (fao.org/faostat; FAO: Cereal Supply and Demand Brief, May 2020). The expected consumption of wheat will reach 858 million tons in 2050 (Alexandratos & Bruinsma, 2012) which will require a 12% increase in production. Therefore, new high-yielding wheat cultivars more resistant to pests and diseases and capable of withstanding climate change will be necessary.

3.1.1 Common wheat

The most widely cultivated wheat species is common wheat or bread wheat (*Triticum aestivum* L.). It has a large (1C = 15.5 Gbp; IWGSC, 2018) and complex allohexaploid genome (2n = 6x = 42) that is composed of three closely related subgenomes A^u, B and D (A^uA^uBBDD). The genome resulted from two successive hybridization events.

About 0.5–0.3 million years ago in the Fertile Crescent, two diploid wheats hybridized resulting in the wild emmer wheat, *T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. et Graebn.) Thell. (A^uA^uBB; Dvořák et al., 1988; Dvořák & Zhang, 1990; Dvořák et al., 1993; Huang et al., 2002).

The diploid *T. urartu* Tuman *ex* Gand. contributed the genome A^u and some undefined species of genus *Aegilops* contributed the genome B (Huang et al., 2002; Dvořák & Akhunov, 2005; Chalupska et al., 2008). *Aegilops speltoides* Tausch is believed to be the B genome donor (Sarkar & Stebbins, 1956; Riley et al., 1958; Zohary & Feldman, 1962; Zhang et al., 2017a).

More than 9000 years ago, *T. turgidum* ssp. *dicoccoides* was domesticated by Neolithic farmers in the Diyarbakir region in today's Turkey (Nesbitt & Samuel, 1996; Özkan et al., 2010). The domestication and cultivation gave rise to at least two distinct lineages of domesticated tetraploids: *T. turgidum* L. ssp. *dicoccum* (Schrank) Thell. (domesticated emmer) and *T. turgidum* spp. *durum* (Schrank) Thell. (domesticated durum or hard/pasta wheat; Pont et al., 2019) Both domesticated subspecies have mutated *Br* loci (Nalam et al., 2006); thus,

they have non-brittle rachises that do not disarticulate before harvest. The major difference between the domesticated emmer and durum is a mutation in *Q* gene (or domestication gene) on chromosome 5AL (Faris & Gill, 2002; Simons et al., 2006) and in *tg2* (tenacious glume gene) on 2BS and 2DS (Simonetti et al., 1999; Jantasuriyarat et al., 2003; Nalam et al., 2007). Compared with the domesticated emmer with the *q* and *Tg2* alleles, the domesticated durum has soft glumes and is free-threshing (seeds are released from rachises at threshing).

Subsequently, about 8000 years ago in the southwestern Caspian Sea region, *T. turgidum* (possibly ssp. *durum*) hybridized with the diploid *Ae. tauschii* Coss. (genome DD) resulting in the hexaploid *T. aestivum* (Kihara, 1944; McFadden & Sears, 1946; Dvořák et al., 2012; Wang et al., 2013; Pont et al., 2019). Nowadays, common and durum wheat account for about 95% and 5% of the global wheat production, respectively (Kadkol & Sissons, 2016).

A small number of hybridization events, that led to bread wheat, limited the resulted gene pool, which was further narrowed down by intensive breeding (Feuillet et al., 2008). Therefore, the amount of alleles for desired traits (such as disease resistance genes) is limited. Wheat's diploid and tetraploid progenitors and other related species (including *Aegilops*, *T. turgidum, T. monococcum,* rye, or *Haynaldia villosa*) are attractive sources of new and desired genes that can be used for the gene pool enrichment (Sando, 1935). The best example is the substitution of the wheat 1AS/1BS chromosomal arms with the rye 1RS chromosomal arm improving resistance to diseases and drought and increasing seed size, which became the base for many cultivars grown around the world (Rabinovich, 1998).

3.1.2 Einkorn wheat

Einkorn wheat (*T. monococcum* L.), literally 'single grain' because domesticated varieties usually produce one grain per spikelet, is a diploid wheat species (2n = 2x = 14; A^mA^m) closely related to *T. urartu*, the donor of the A genome of common wheat. Einkorn has two morphologically different forms (Tutin et al., 1980), the domesticated form *T. monococcum* L. and the wild form *T. boeoticum* Boiss. emend. Schiem (Schiemann, 1948). The main differences between *T. boeoticum* and *T. monococcum* are shown in Figure 1.

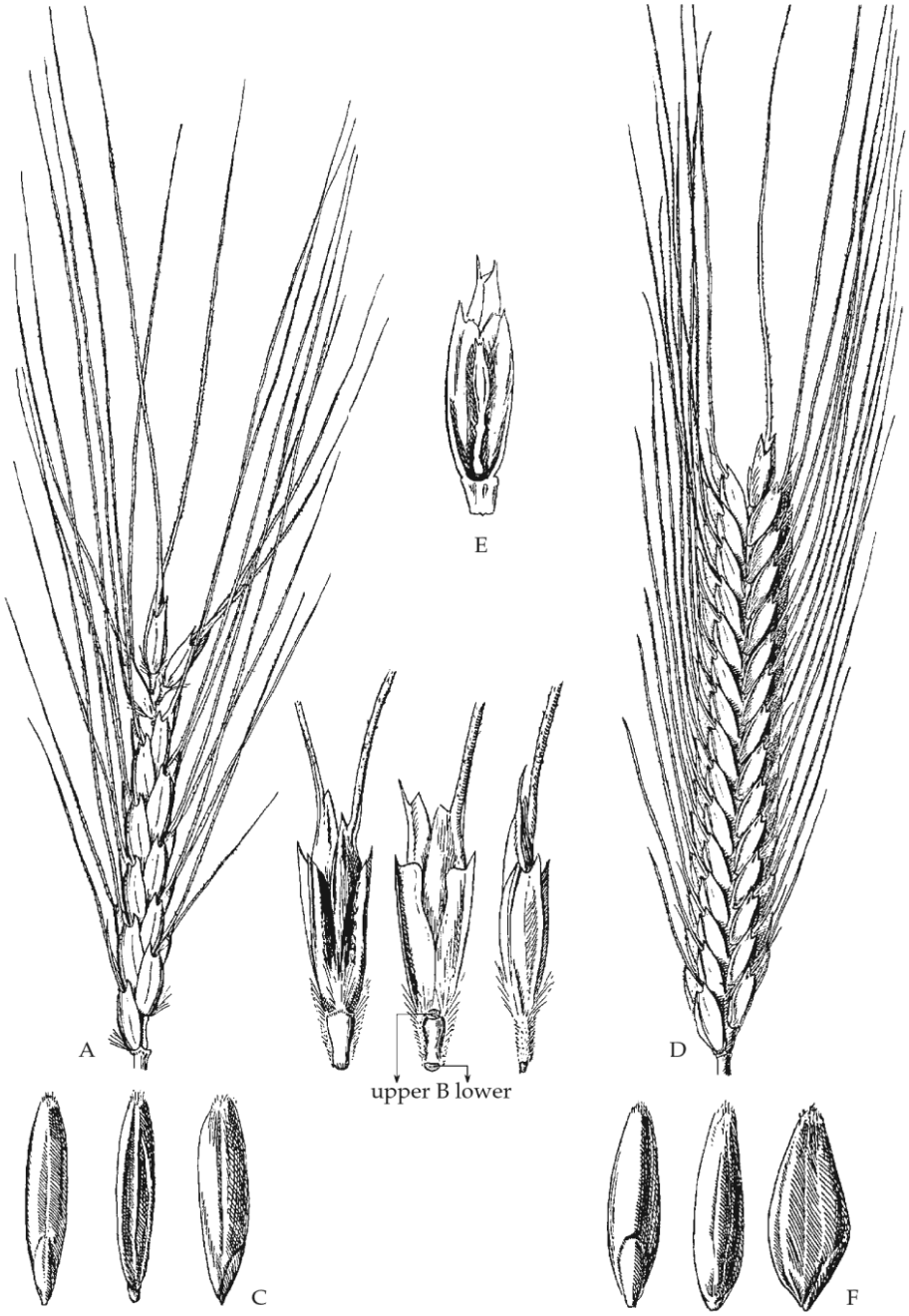


Figure 1. The difference between wild and domesticated einkorn wheat.

Wild einkorn *Triticum boeoticum* Boiss. emend. Schiem.: **A)** ear, **B)** shattering spikelet with upper and lower disarticulation scar, and **C)** grain. Domesticated einkorn *Triticum monococcum* L. ssp. *monococcum*: **D)** ear, **E)** non-shattering spikelet, and **F)** grain (Schiemann, 1948).

The domesticated einkorn was derived from *T. boeoticum* through the acquisition of a non-brittle rachis more than 10 000 years ago in the Turkish Karacadağ Mountains (Dvořák et al., 1988; Tanno & Willcox, 2006; Haldorsen et al., 2011; Brandolini et al., 2016). *T. monococcum* is not free-threshing as it has tough glumes, which do not allow the grains to be easily released from the spikelets (Taenzler et al., 2002; Sood et al., 2009). It is a relatively low-yielding crop but can survive on poor soils where other wheats cannot. It was among

the first cultivated cereals and an important source of food, but its popularity gradually decreased during the Bronze Age when high-yielding free-threshing wheats became cultivated more extensively (Harlan & Zohary, 1966; Heun, 1997; Zohary et al., 2013). Einkorn was then left growing in its natural habitats without selection for thousands of years. Nowadays, einkorn is just sporadically grown in mountainous areas (Zaharieva & Monneveux, 2014). Its flour has poor rising qualities; thus, einkorn has been usually consumed as cooked whole grains or porridge. However, the flour is rich in protein, resistant-starch, fiber, minerals, and phytochemicals (carotenoids, flavonoids, phytosterols, and phenolic compounds) and has been recently 'rediscovered' by the organic food industry in Europe (Arzani & Ashraf, 2017). Nevertheless, there is just a little to no added nutritional value from consuming einkorn instead of bread wheat for a healthy person with a balanced diet (Van Boxstael et al., 2020).

The availability of both domesticated and wild forms is the reason why there is a greater variability compared to common wheat (Kilian et al., 2007). Coupled with einkorn's diploid genome and collinearity with other *Triticum* species (Dubcovsky et al., 1996; Marino et al., 2018), it makes it an ideal model for agronomically important gene mapping (Yu et al., 2019), gene cloning (Yan et al., 2003; Yan et al., 2004), or functional gene validation (Loukoianov et al., 2005).

Wheat breeding programs are mainly focused on high and stable yield, and resistance to biotic and abiotic stress factors (Lumpkin, 2015). Yield is a result of expression of multiple traits that can be qualitative or quantitative in nature. It is also strongly influenced by the environment. This causes low heritability of yield *per se* and makes it more challenging to be improved (Wu et al., 2012). However, yield can be divided into less environmentally sensitive yield components: number of plants per square meter, spike number per plant, grain number per spike, and grain weight (Cuthbert et al., 2008). These main components are directly or indirectly affected by many other related traits such as plant height, the number of tillers, plant shape, pubescence, spike length, spikelet number per spike, spikelet fertility, flowering time, the number of grains per spikelet, and grain size. Many of them exhibit high heritability; therefore, it is useful to focus on single components when trying to improve yield as a whole (Slafer, 2003).

3.2 Leaf pubescence

In light of global warming and climate change, leaf pubescence becomes one of the traits that could help breeders develop new cultivars better adapted to stress conditions. Trichomes are specialized plant structures extending from the epidermal layer of aerial tissue that vary greatly in size, shape, number, composition, location, and function (Levin, 1973). The presence of epidermal trichomes on different plant organs, including leaves, stems, flowers, and seeds, is a useful phenotypic character that is widely spread in the plant kingdom. They are usually classified into two main types: glandular and non-glandular. Non-glandular trichomes provide mechanical protection against unfavorable weather, herbivores, and pests. The effects depend on density, length, erectness, and shape of trichomes. Whereas glandular trichomes produce, store, and secrete different types of secondary metabolites (Fahn, 2000; Glas et al., 2012) that provide protection against phytopathogens and herbivores and limit water evaporation and damage caused by UV irradiation. Some of the commonly found plant metabolites are terpenoids (Gershenzon et al., 1992; Gershenzon & Dudareva, 2007), methyl ketones (Dimock & Kennedy, 1983), phenylpropanoids (Deschamps et al., 2006), flavonoids (Voirin et al., 1993), and acyl sugars (Weinhold & Baldwin, 2011). Many of them are commercially important (Schilmiller et al., 2008).

Leaf pubescence is linked to drought tolerance because a layer of trichomes creates a microclimate that reduces the air movement and therefore transpiration (Ghorashy et al., 1971) and helps to accumulate water (Konrad et al., 2014). A dense layer of trichomes also increases spectral reflectance within the range of 400–700 nm; therefore, it reduces net radiation and leaf temperatures. Moreover, it protects cells from damage by UV radiation (Karabourniotis et al., 1995; Tattini et al., 2000). In low-temperature environments, the trichomes protect the layers underneath from frost. Some plants can tolerate high levels of heavy metals in the soil through trichome secretion of metal compounds (Choi et al., 2001; Sarret et al., 2006). For a review of the influence of leaf pubescence in plant-environment interactions, see Bickford (2016).

Trichomes also provide mechanical protection against small herbivores like insects, because they find it difficult for their feeding organs to reach the leaves (Cardoso, 2008). Even larger herbivores can be put off as stiff trichomes irritate their palates. Glandular trichomes produce metabolites that can be sticky and toxic; thus, both glandular and non-glandular trichomes affect attachment, movement, shelter, feeding, digestion, oviposition, and viability of insects (Webster, 1975; Norris & Kogan, 1980; Simmons et al., 2004).

3.2.1 Leaf pubescence in wheat

In wheat, trichomes can be found on glumes, nodes, leaf sheath, and blade. There is a great variability of leaf trichome density and length among the *Triticum* species (Pshenichnikova et al., 2016). The pubescence of hexaploid wheats varies greatly, e.g., *T. aestivum* ssp. *spelta* and *T. aestivum* cv. 'Chinese Spring' have a very low number of short trichomes (~30 μm), whereas cv. 'Hong-mang-mai' and 'Saratovskaya 29' have a moderate number of long trichomes (~150 μm; Pshenichnikova et al., 2016); thus, while they all have straight non-glandular unbranched unicellular trichomes (Figure 2), the trichome density and length are characteristic of a cultivar. Drought-resistant cultivars have a higher density of trichomes, which positively influence water retention, whereas cultivars grown in humid climate have only weak pubescence (Pshenichnikova et al., 2018). Therefore, leaf pubescence could be one of the important targets in breeding drought-resistant cultivars.

Figure 2. A leaf trichome of common wheat.

Colored scanning electron micrograph by Stefan Diller. Reprinted with the author's permission. Scale bar = 100 μm.

Besides the protection against abiotic stresses, leaf trichomes provide resistance to certain insects. Greater hair density and hair length is responsible for a significant reduction in the number and viability of laid eggs and larval growth and survival of cereal leaf beetle (*Oulema melanopus* L.; Gallun et al., 1966; Schillinger & Gallun, 1968). Trichomes also provide protection against hessian fly in common wheat (*Mayetiola destructor* Say; Roberts et al., 1979) but, interestingly, not in diploid wheat (Sharma et al., 1992). However, the pubescent wheats were found to be more susceptible to air-borne wheat curl mite (*Aceria tosichella* Keifer) which is the only known vector of wheat streak mosaic virus (Slykhuis, 1955). Therefore, the occurrence of this virus is higher on pubescent than on glabrous wheats because of the more favorable landing site facilitated by the leaf hairs (Harvey & Martin, 1980).

The inheritance of leaf pubescence in wheat has not been clearly explained yet. The phenotypic analysis of mapping populations has been problematic because some methods are less accurate (counting with optical magnification) or efficient (scanning electron microscopy) but computational analysis of digital images has solved some of the issues since then (wheatdb.org/lhdetect2; Genaev et al., 2012). The density of leaf trichomes is different both at the seedling and the adult stage and is variable on different leaves within lines. It might be controlled by different genes and affected by many modifiers at each of the stages (Maystrenko, 1976; Taketa et al., 2002).

Several genes controlling wheat leaf pubescence have been mapped in **common wheat**: *Hl1* (*leaf hairiness 1*, syn. *Pa*; Maystrenko, 1976) on chromosome 4BL (Dobrovolskaya et al., 2007), *Hl2* on 7BS (Taketa et al., 2002), and QTLs for hairy leaf margins and auricles on chromosomes 4BL and 4DL (Dobrovolskaya et al., 2007). Later, additional loci were detected on 7A (Shahinnia et al., 2013, as cited in Doroshkov et al., 2015) and 7D (Doroshkov et al., 2014); therefore, Doroshkov et al. (2015) suggest a presence of a homoeologous series of genes on chromosomes of the 7th homoeologous group of *T. aestivum*.

Hl2^{aesp} introgressed from *Ae. speltoides* was mapped on chromosome 7BS in the wheat/*Aegilops speltoides* introgression line 102/00^{i} (Pshenichnikova et al., 2006; Dobrovolskaya et al., 2007), and a QTL associated with leaf sheath hairiness introgressed from *Ae. speltoides* was mapped on 4DL in synthetic hexaploid wheat (Wan et al., 2015). Interestingly, the QTL on 4DL appeared to be in a tight linkage with a QTL for grain yield and grain weight (Wan et al., 2015).

Another gene detected by Maystrenko (1976) and Dobrovolskaya et al. (2007) has not been localized yet; thus, Doroshkov et al. (2015) temporarily named it *Hl3*, and described a model of how *Hl1*, *Hl2*^{aesp}, and *Hl3* affect the phenotype: both *Hl1* and *Hl3* seem to positively regulate trichome initiation and growth, while *Hl2*^{aesp} is responsible for the growth of longer trichomes. Their effects appear to be independent and other genes must be controlling the trait because recessive homozygotes still have slightly pubescent leaves. Therefore, *Hl* genes seem

to be controlling the intensity of hairiness rather than the presence-absence (Taketa et al., 2002; Dobrovolskaya et al., 2007).

Besides genes controlling leaf pubescence, a gene for glume pubescence (*Hg*; Khlestkina et al., 2002) and a QTL for node pubescence (*Hn*; Sourdille et al., 2002) were mapped on 1AS and 5AL, respectively.

Genes controlling pubescence were identified in other cereals as well. In **barley** (*Hordeum vulgare* L.), a gene for leaf blade pubescence (*Pub*) was mapped on 3HL (Pickering et al., 1997), a gene for hairs on lemma nerves/hairy leaf sheath (*Hln/Hsh*) together with QTLs controlling leaf sheath pubescence were mapped on 4HL (Takahashi & Hayashi, 1966; Saade et al., 2017), a gene for large trichomes (*Ltc/Ltr*) was mapped on 5H (Franckowiak, 1997), and a gene for glume pubescence (*pbg*) was mapped on 7H (Hor, 1924, as cited in Franckowiak, 1997). *Hsh*$_b$, derived from *Hordeum bulbosum* L., is allelic to *Hln/Hsh* and homologous to the gene for peduncle and leaf sheath pubescence *Hp1* on 5RL in **rye** (*Secale cereale* L.; Pickering et al., 1997; Korzun et al., 1999; Cockram et al., 2010).

Dobrovolskaya et al. (2007) reported that genes and QTLs on 4BL and 4DL (*Hl1* and QTLs for leaf margins and auricles pubescence) are colinear to genes for barley pubescence *Hln*/*Hsh* and *Hs*$_b$ on 4HL (Korzun et al., 1999) and for rye pubescence *Hp1* on 5RL (Devos et al., 1993; Korzun et al., 1996); thus, the locus might be pleiotropically controlling the pubescence of different plant organs in different *Triticeae* species.

Diploid wheat *T. boeoticum* has pubescent leaves, while domesticated *T. monococcum* is glabrous (Tutin et al., 1980). The F_1 hybrids of a cross of *T. boeoticum* and *T. monococcum* are pubescent and the F_2 plants segregate into 1 densely pubescent : 2 intermediate : 1 glabrous (meaning that leaf pubescence is dominant over leaf glabrousness). However, as Sharma & Waines (1994) noticed, there is a wide range of trichome density and length within intermediate and densely pubescent classes. Thus, if scored in terms of the presence-absence of trichomes, the trait appears to be controlled by one dominant gene, but if the character is scored on the actual number and length, the trait seems to be controlled by a polygene. The trait was reported to be controlled by two dominant genes without chromosome assignment (Smith, 1936 and Smith, 1939, as cited in Sharma & Waines, 1994), by one dominant gene without chromosome assignment (Kuspira et al., 1989; Sharma & Waines, 1994), by one dominant gene on $5A^mL$ (Jing et al., 2007; Jing et al., 2009), and by one QTL on $5A^mL$ (Hori et al., 2007; Yu et al., 2016).

3.3 Quantitative trait loci

Agriculturally important traits, such as tolerance to biotic and abiotic stress factors, or yield and quality are very often a result of more than one gene effect and are called quantitative traits (also complex, continuous, or polygenic traits). The phenotypic variation of quantitative traits is continuously distributed in natural populations. The genetic variation of a quantitative trait is controlled by the collective effects of multiple genes (a polygene) and their potential interaction with the environment (e.g., sunlight, temperature, rainfall, humidity, and soil degradation). Non-genetic quantitative traits are not controlled by genetic factors and their variation in a population is caused only by environmental factors, while genetic quantitative traits are affected by both genetic and environmental factors (Chen, 2014). The effect of each of the multiple genes is usually relatively small. The effects are also influenced by the individual genetic background and sex.

The genomic regions containing genes contributing to the variability of the quantitative traits are called **quantitative trait loci** (QTLs). A single quantitative trait locus (QTL) might be a single gene or a cluster of linked genes. QTLs could be either minor or major. Minor QTLs explain less than 10% of the total phenotypic variation while major QTLs explain more than 10%. In extreme cases, major QTLs can be treated as qualitative traits (Chen, 2014).

Besides the interaction of genes with the environment, the genes themselves interact with each other. Usually, a phenotype of a quantitative trait is a sum of the effects of individual QTLs. However, an individual QTL might not have its own effect, but together with other QTLs, it contributes to the phenotype as a net effect. This deviation from the sum of the independent effects of the individual genes is called epistasis and these QTLs are **epistatic QTLs** (Falconer & Mackay, 1996). Some QTLs are **pleiotropic**, which means that they affect more than one trait. If more QTLs for different traits cluster within a small genomic region, it might be caused by the presence of a single pleiotropic QTL (Peng et al., 2003). Pleiotropic QTLs should not be mistaken with two or more linked QTLs that affect different traits. That is difficult because in linkage mapping studies, the intervals to which QTL maps usually contain multiple genes.

The gene expression levels can be modified by polymorphisms in regulatory loci and therefore treated as quantitative traits. These loci are called **expression QTLs** (eQTLs). Regulatory genes responsible for the variability in the expression of a specific protein are described as **protein quantity loci** (pQLs). Similarly, **metabolite QTLs** (mQTLs) can be mapped by measuring the abundance of a specific metabolite in all lines of a mapping population and using it as a phenotypic trait. The combined analysis can lead to a better understanding of the regulatory network of genes (Wang et al., 2014).

Identification of agronomically important traits in wheat and their further exploitation in crop breeding requires mapping of the corresponding QTLs in the crop genomes. Two main approaches are available: linkage-based mapping and association mapping.

3.4 Linkage-based mapping of quantitative trait loci

QTL mapping is a statistical analysis that allows finding of genomic regions that affect the trait of interest and describing their contribution to trait variability. It uses segregating mapping populations (derived from a cross of parental lines that are contrasting for the trait of interest) and their linkage maps. The mapping resolution depends on the number of recombinations in the mapping population. Higher mapping resolution and precision require larger mapping populations (Members of the Complex Trait Consortium, 2003).

For a reliable assessment of mapped QTLs and their contribution, QTL-environment effects need to be minimized. Interactions with the environment may manifest as QTL effects detected only in a subset of the total number of environments, significant changes in the magnitude of QTL effects across environments, and opposite favorable alleles at a QTL in specific environments (Hayes et al., 1993). QTL analyses performed in multiple replicates reduce environmental effects and increase statistical power. Additionally, by replicating the trials in multiple environments and over time, it becomes possible to estimate QTL-environment interactions.

Linkage-based mapping uses the principle that QTLs can be traced down via their genetic linkage to marker loci or interval that can be readily classified. Generally, a linkage map, that provides the positions of markers on all chromosomes of the genome and the genetic distances between them, is required.

3.4.1 Linkage maps

Genetic maps are an essential tool in genetics and breeding. Maps can be divided into two types: linkage maps and physical maps. Linkage maps are based on recombination frequencies between genetic markers that form linkage groups showing their order and distances. Physical maps show the physical locations of DNA sequences of interest with distances typically measured in base pairs. The most advanced physical maps are whole genome sequences.

Linkage maps facilitate QTL mapping, gene cloning, marker-assisted selection, and comparative genomics. Linkage mapping is used to determine a relative linear order and distances of molecular markers and to assign them to particular chromosomal regions. Molecular markers rely on polymorphisms between parental lines. Polymorphisms can be detected using markers including restriction fragment length polymorphism (RFLP; Botstein et al., 1980), random amplified polymorphic DNA (RAPD; Williams et al., 1990), amplified

fragment length polymorphism (AFLP; Vos et al., 1995), cleaved amplified polymorphic sequence (CAPS; Neff et al., 1998), sequence-tagged site (STS; Olson et al., 1989) or expressed sequence tag (EST; Adams et al., 1991), simple sequence repeats (SSR; Hearne et al., 1992), and single nucleotide polymorphism (SNP).

During meiosis, homologous chromosomes pair and recombine at various positions, which is the basis for linkage mapping. Therefore, markers are ordered, and genetic distances are calculated based on the amount of meiotic recombination that occurs between them (Sturtevant, 1913). In general, the probability that recombination occurs between two markers on a chromosome depends on their physical distance. The nearer the markers are located to each other, the more they will tend to co-segregate together during meiosis. With an increasing distance between them, the probability for recombination increases as well and genetic linkage gets weaker. Therefore, genetic linkage can be interpreted as a measure of physical distance.

The relationship between recombination fraction and genetic distance is approximated by mapping functions. A mapping function relates recombination fraction and genetic distance in units of Morgans (M), or more commonly used centiMorgans (cM; 1 M = 100 cM), named in honor of Thomas Hunt Morgan. 1 cM represents a recombination frequency of 1%. Various mapping functions are available, e.g., Haldane mapping function (Haldane, 1919), Kosambi mapping function (Kosambi, 1943), Carter-Falconer mapping function (Carter & Falconer, 1951), and Felsenstein mapping function (Felsenstein, 1979). The latter three capture a certain degree of crossover interference, which refers to a situation in which the occurrence of a crossover at a locus affects either positively or negatively the chance of a second crossover in the vicinity of the locus. With a high number of markers, the computation of marker order and distances becomes complicated; therefore, algorithms and programs have been developed based on maximum likelihood (Morton, 1955). There are many programs to determine the order of markers and their distances, e.g., MapMaker (Lander et al., 1987), JoinMap (Stam, 1993), MapManager (Manly et al., 2001), MultiPoint (Mester et al., 2003), R/qtl (Broman et al., 2003), and IciMapping (Meng et al., 2015). Linked markers are grouped into linkage groups, that represent chromosomal segments or entire chromosomes.

The resolution of a linkage map depends on the number of recombinations scored in an experimental population. Recombinations are not evenly distributed across the chromosome as recombination is suppressed around the centromeric regions. This affects the resolving power of linkage analysis, so markers that are physically far apart may appear at the same position on the map. The size of mapping populations determines the resolution of linkage mapping. About 80–120 lines are used to construct an initial genetic map. This provides an acceptable level of precision, while the cost and labor are still manageable. However, in order to separate markers much closer to the target gene for map-based gene cloning, thousands of

individuals are used to get the required level of precision (Tiwari et al., 2016a). Multiple types of mapping populations can be used for linkage map construction and QTL mapping.

3.4.2 Mapping populations

The first step in linkage map construction for linkage-based QTL analysis is the preparation of biparental mapping populations that are developed from a cross of two divergent lines selected from either natural or mutant populations. Some types of mapping populations are more suitable for QTL mapping than the others.

The simplest type of biparental mapping population is an **F_2 population**. Two homozygous inbred plants or doubled haploid plants are selected as parents. It is desirable to obtain contrasting parental lines in all traits of interest. The polymorphism can be evaluated both at the phenotypic level and by molecular markers. Due to Mendel's law of dominance and uniformity, all plants of the F_1 generation will have the same genotype and similar phenotype. The F_1 population is then self-pollinated (or sib-mated in the case of allogamous species) and the F_2 population segregates for the traits of interest. The number of recombinations is limited because F_2 populations are the outcome of a single meiosis. Another disadvantage is that F_2 populations can hardly be preserved because F_3 lines are not identical to F_2 lines. Thus, they cannot be used for repeated experiments under various environments (Acquaah, 2012). Compared with recombinant inbred lines, doubled haploid lines, and near-isogenic lines, F_2 populations are less suitable for QTL mapping because genes are not yet fixed and only QTLs with large effects and stable expression can be detected (Tian et al., 2015).

Recombinant inbred lines (RILs) are obtained by a single-seed descent method, i.e. by repeated selfing of the same lines for at least 6 generations. At that point, less than 2% of the genome remains in heterozygotic composition and further segregation in the progeny of RILs is insignificant. In comparison with F_2 or doubled haploid lines, the degree of recombination is higher because RILs go through more rounds of meiosis. RILs are useful for high-resolution genetic mapping and ideal for QTL mapping. They are permanent and can be easily used in replicates. However, their preparation is time-consuming (Schneider, 2005; Acquaah, 2012).

Doubled haploid lines (DH lines) have two identical sets of chromosomes in their nuclei. Therefore, they are utterly homozygous. DH lines are developed by androgenesis (microspore and anther culture), gynogenesis (ovary and ovule culture), or wide hybridization (pollination of the F_1 floret with maize pollen and embryo rescue), followed by colchicine treatment (Laurie & Bennett, 1988). The wide hybridization method is considered to be the most efficient one (Niu et al., 2014). DH lines are used for high-resolution QTL mapping as their genetic structures represent the segregations and recombinations of alleles during F_1 gamete formation, but the recombination is limited similarly to F_2 populations because there is only one round of meiosis (Tian et al., 2015). Thus, the preparation of DH lines is timesaving,

but genetic mapping using DH lines cannot reach the same resolution as with RILs. Similarly to RILs, DH lines are permanent.

Backcross (BC) populations are used to analyze specific DNA fragments from a donor in the genetic background of a recipient. A hybrid F_1 plant, the donor, is backcrossed to the recipient. During meiosis, unlinked donor DNA fragments are separated by segregation while linked donor fragments are reduced because of recombination. Compared to F_2 populations, BC_1 populations provide better QTL mapping efficiency, but they cannot be maintained for the long term; therefore, their usage in QTL mapping is limited (Tian et al., 2015).

The amount and size of donor fragments in BC lines are reduced with every round of backcrossing. After several rounds, the BC lines and the recipient differ only in one chromosomal fragment. Such BC lines are called **near-isogenic lines (NILs)**. NILs allow to Mendelize the quantitative traits (such that only the QTL of interest segregates) affecting the same trait, so the interference derived from the genetic background and the masking effects of major QTLs over minor QTLs are removed (Schneider, 2005; Tian et al., 2015). NILs are permanent as well.

Besides the mapping populations mentioned above, there are plenty of other designs, e.g., residues of alloplasmic lines (RHLs), QTL isogenic lines (QIRs), single-segment substitution lines (SSSLs), and chromosome segment substitution lines (CSSLs; Tian et al., 2015).

3.4.3 Statistical methods of quantitative trait loci mapping

QTL mapping is an analysis aimed at detecting and then locating QTLs. Measurements of phenotypic traits need to be obtained for all individuals in the mapping population. Once a linkage map is constructed and the population is phenotyped, it is possible to proceed with the QTL mapping. Statistical methods are used to assess the correlation between the values of the phenotypic trait of all individuals and different marker locus genotypes.

Many statistical software packages can be employed, e.g., MapMaker/QTL (Lincoln et al., 1993), MultiQTL (Korol et al., 2001), R/qtl (Broman et al., 2003), QGene (Joehanes & Nelson, 2008), QTLCartographer (Wang et al., 2012a), and IciMapping (Meng et al., 2015). Several statistical methods of linkage-based QTL mapping are available, some of them are outlined below.

The simplest and oldest one is a **single-marker analysis** that tests the marker's statistical influence on the trait by finding a relationship between markers and phenotype. A variety of statistical analyses is available, including *t*-test (Gosset, 1908), analysis of variance (ANOVA; Fisher, 1918), or regression (Kearsey & Hyne, 1994). Single-marker analysis tests the marker's linkage to a QTL one at a time. Therefore, the advantage is that a complete genetic

map is not needed, and the computation is relatively straightforward and possible to do using statistical software like Microsoft Excel (Microsoft Corp.). The disadvantages are low detection power and accuracy of QTL positions relative to markers. Also, close weak QTLs and distant strong QTLs cannot be told apart (Chen, 2014).

For **simple (or single) interval mapping (IM)**, a constructed linkage map is required. IM is based on maximum likelihood parameter estimation and provides a likelihood ratio test for a QTL position between a pair of adjacent mapped (flanking) markers to detect a QTL in between them. Genetic distances between the markers are utilized to explore loci between the markers which is not possible using single-marker analysis. IM method supposes that there is only one QTL over the whole genome for the studied trait, but quantitative traits are usually affected by many QTLs. Therefore, all QTLs, except the strongest one, are ignored. IM is not effective for complete QTL models because each QTL is taken into consideration one at a time and logarithm of the odds ratio (LOD score) is calculated like it is the true QTL. Thus, QTLs outside the interval can interfere and compromise the positions and effects of QTLs within the interval (Lander & Botstein, 1989). A similar approach uses regression mapping instead of ML (Haley & Knott, 1992) but both still have issues with separating multiple QTLs on the same chromosome (Martínez & Curnow, 1994).

Composite interval mapping (CIM) is a method that combines IM with multiple marker regression; thus, it detects QTLs in multiple intervals using multiple marker information. In comparison with IM, other interacting QTLs are taken into consideration and variation associated with them is removed using additional cofactor (background) markers outside the window of analysis (Jansen, 1994; Zeng, 1994). An improved version of CIM is called **inclusive composite interval mapping (ICIM)**. It employs stepwise regression to select significant cofactor markers and estimate their corresponding effects. After that, the phenotypic values are adjusted by all markers retained in the regression equation except the pair of markers flanking the current scanning position. The adjusted phenotypic values are then used in interval mapping. Compared with CIM, ICIM has increased detection power and precision and removes the arbitrariness of cofactor selection (Li et al., 2006).

Multiple interval mapping (MIM) assumes the presence of one or more QTLs in the genome and accommodates interaction effects among QTLs, i.e. reduces the residual variation (while previous one-dimensional QTL mapping methods suppose that only one QTL and additive effects are present). MIM creates a CIM model and adds and removes QTLs by stepwise selection. Then, an iterative expectation-maximization algorithm estimates the QTL likelihoods and searches for epistatic effects between them (Kao & Zeng, 1999; Zeng et al., 1999).

An output from interval mapping is usually a graph with markers and their genetic distances on the *x*-axis and corresponding LOD (logarithm of the odds) scores on the *y*-axis. A significance threshold can be visualized as well.

A **LOD score** is traditionally used for summarizing the evidence for a QTL. It is the log base 10 of the ratio of the maximum likelihoods under the alternative (a linkage is present) and null hypotheses (no linkage), which indicates how much more probable the data are to have arisen assuming the presence of a QTL than assuming its absence (Morton, 1955). Traditionally, the QTLs are considered to be significant above the threshold LOD score 3 (Lander & Botstein, 1989), which means that the odds that the QTL is present are 1000 times greater than the odds that it is absent (it corresponds to a genome-wide false positive rate in the neighborhood of 5%; Lander & Kruglyak, 1995). However, significance thresholds in QTL mapping are usually obtained by permutation tests (Fisher, 1935) as proposed by Churchill & Doerge (1994). Permutation tests maintain the linkage maps with marker genotypes but shuffle the values of the phenotypic traits across the experiment lines. After that, the same QTL mapping methods are applied to the shuffled data to reveal the level of false-positive marker-trait associations. This is repeated at least 1000 times and the resulting threshold is given based on the level of false positives (Chen, 2014). Another resampling method that is widely used is bootstrapping. The phenotypic traits are shuffled as well but with replacement such that after an experiment line receives a random trait assignment, some other line may receive the same random trait assignment. Thus, the difference is that the permutation test keeps the summary information of the trait while bootstrapping changes the mean and variance (Doerge, 2002).

Markers linked to a QTL segregate together which is disrupted by recombination and only closely linked markers eventually remain in proximity of the locus. To precisely localize a QTL, individuals in which recombination took place in the proximity of the QTL are needed because then only closely linked markers remain linked. For high-resolution mapping (or fine-mapping; in the intervals of 0.1–1 cM), thousands of individuals and high marker density are needed. Therefore, QTL mapping usually localizes the QTLs in broad intervals (10–20 cM) using a sparse skeletal (or framework) map and then the chromosomal regions containing the QTLs are further narrowed down (Mackay et al., 2009). This is done by using individuals in which recombination occurred between the markers flanking (surrounding) the QTL. This part needs time-consuming breeding of more individuals to acquire the required recombination and designing markers within the region.

3.4.4 Verification of quantitative trait loci and identification of genes

When significant QTLs are mapped, it is essential to verify them by doing a replication study. That is a QTL analysis on an independent population made by a cross of the same parental genotypes, closely related genotypes, or important cultivars. Nevertheless, unverifying QTLs

does not necessarily mean that they are false positives because linkages often involve weak effects that may not manifest in replication studies (Lander & Kruglyak, 1995; Mackay et al., 2009). Then, once chromosomal regions are verified, it is possible to identify the genes.

QTL mapping is just the first step in the process of identifying the genes controlling a quantitative trait. The results of high-resolution QTL mapping are sufficient for selective breeding programs (through marker-assisted selection) but not for identifying alleles at a locus responsible for the difference in phenotypes, which is essential for describing the genetic basis of quantitative variation and application of transgenic technology to agronomically important traits.

Positional cloning through high-resolution mapping is used for the identification of genes explaining the observed QTL. It provides a reduced number of candidate genes for validation analysis and it is feasible for loci defined by mutant alleles of large effects (Falconer & Mackay, 1996). The increase in mapping resolution can be achieved by producing a new large mapping population derived from a cross of two NILs differing only for the alleles at the target QTL which is then considered to be Mendelized (segregating in 1:2:1 or 1:3 ratios). Flanking markers are used for anchoring the genetic map to the physical map (a genomic sequence or at least a contig containing the QTL region). New markers are then designed for map saturation and candidate genes identification.

The validation of candidate genes can be done through both forward and reverse genetic approaches, e.g., complementation test (Liu et al., 1999), insertional mutagenesis using T-DNA and transposable elements (Maes et al., 1999; Meissner et al., 2000), RNA interference (Waterhouse et al., 1998; Waterhouse & Helliwell, 2003; Kusaba, 2004), homologous recombination-mediated gene transfer (Hanin & Paszkowski, 2003), TILLING (targeting induced local lesions in genomes; McCallum et al., 2000; Chen et al., 2012), VIGS (virus-induced gene silencing; Baulcombe et al., 1999; Liu et al., 2002; Hosseini Tafreshi, 2011), and CRISPR/Cas9 (Jinek et al., 2012; Cong et al., 2013; Mali et al., 2013).

3.5 Association mapping

Alternatively, QTLs affecting the trait variation can be identified using natural populations by association mapping (AM). AM is based on linkage disequilibrium (LD), which relates to a non-random association (distorted segregation) of alleles at different loci. Two loci are in LD when an allele at one locus is found together with an allele at a second locus more often than expected if alleles were combining independently. AM searches for an association between allelic variants at marker loci and the mean of the trait within a set of unrelated genotypes. The marker-trait association indicates that there is a connection between the genes controlling the trait and the marker locus (Flint-Garcia et al., 2003). The number of markers needed for AM depends on the scale and pattern of LD. It is necessary to genotype just one marker in the block

as a proxy for the rest of them. As the size of LD blocks increases, the total number of required markers decreases (Mackay et al., 2009). AM takes advantage of associations created in the relatively distant past. Many generations have elapsed since then; therefore, recombination has removed the association between QTLs and markers not tightly linked to them. Thus, AM allows for much finer QTL mapping than traditional linkage-based QTL mapping.

Multi-parent populations developed by the crossing of multiple parents, multiple biparental populations, breeding pools, or diverse natural populations can be used for the identification of QTLs by AM. A set of diverse genotypes is thus used to create an association panel, which covers most of the variability available in a gene pool for desired traits. Therefore, more alleles at a locus take part in the detection of a QTL instead of two alleles in biparental populations that have the disadvantage of reduced genetic diversity. Therefore, AM can effectively utilize conserved natural genetic diversity of worldwide crop germplasm resources.

AM uses diverse heterogeneous populations; therefore, smaller populations are needed for QTL mapping because they contain more recombinant individuals. However, this heterogeneity leads to a problem with a specific population genetic structure. Since the population is a combination of diverse lines with different pedigree relationships, subpopulations vary in allele frequencies at many loci and mean trait values. This can lead to false detection of marker-trait associations, but it is possible to reduce the false-positive rate with statistical methods (Yu et al., 2005; Larsson et al., 2013). AM can identify phenotypic effects of alleles with relatively high frequency in the population. Thus, linkage-based mapping is more suitable for rare alleles which can be hardly assessed by AM unless they have large penetrance and significant effects (Álvarez et al., 2014).

The problems with rare alleles and population structure may be solved by some specialized types of populations, such as multiparent advanced generation inter-cross (MAGIC) populations (Cavanagh et al., 2008) and nested association mapping (NAM) populations (Yu et al., 2008). In a MAGIC population, 4, 8, or 16 parents are crossed in biparental fashion and then the F_1 hybrids are crossed in subsequent generations followed by few rounds of selfing. In the NAM population, multiple diverse founder parental inbred lines are crossed with one common parental inbred line followed by several rounds of selfing.

Two main approaches of AM are genome-wide association studies (GWAS) and candidate gene-based approach (Sehgal et al., 2016). GWAS requires a substantial number of densely distributed molecular markers across the genome to test association for various quantitative traits. Whereas the candidate gene approach requires a more complex understanding of the trait of interest, i.e. the selection of markers depends on previous QTL studies or biochemical and regulatory pathways (Lander & Schork, 1994; Pflieger et al., 2001).

Linkage-based QTL analysis and AM are complementary to each other. Linkage-based QTL mapping identifies large chromosomal regions with a rather low marker coverage

and has an increased power to detect QTLs as all segregating alleles are at an intermediate frequency. Since the resolution of linkage maps used for locating QTLs is on the order of 5–10 cM, each interval with a detected QTL can contain several hundreds of genes (Buckler & Thornsberry, 2002; Mackay et al., 2009). On the contrary, AM needs prior information about candidate genes or a genome-wide scan with a high marker coverage but provides precise locations of QTLs with high resolution (Fulker et al., 1999). Linkage-based QTL mapping and AM can be simultaneously combined into one approach called joint linkage association mapping (JLAM) which can be even more efficient for QTL mapping. JLAM uses random parents from a natural population and their F_1 offspring to assess the association of different markers in the original population and co-transmission of marker alleles in a Mendelian fashion from parents to their offspring (Wu & Zeng, 2001; Wu et al., 2002).

Since both linkage-based QTL mapping and AM are time-consuming and expensive, a modification called **selective genotyping** might be used. It is based on the genotyping of selected individuals with extreme phenotypes of one chosen trait. Only these individuals are used for the construction of a linkage map and interval QTL mapping (Lander & Botstein, 1989). This decreases the price of genotyping. The disadvantage is that the effects of QTLs cannot be properly assessed. Multiple strategies are available, such as truncation selection (Slatkin, 1999), truncation selection II, and extreme rank selection (Chen et al., 2005).

An alternative to linkage-based QTL mapping and AM called **QTL-seq** is a recently adopted next-generation sequencing (NGS) based strategy that allows a rapid high-resolution genome-wide QTL mapping. One of the approaches is a variation of bulked segregant analysis (Michelmore et al., 1991). It is based on selecting two groups of 20–50 individuals with extreme phenotypic values from the mapping population, resequencing them with a sufficient genome coverage and then identifying the QTLs by counting and comparing the index SNPs between the two groups (Takagi et al., 2013; Das et al., 2015).

4 Materials and methods

4.1 Biological material

- *Triticum monococcum* L. mapping populations:
  - A mapping population that comprised 81 F_{12} recombinant inbred lines (RILs) developed from a cross of 'DV92' and 'G3116' – DV92 is a cultivated *T. monococcum* ssp. *monococcum* from Titograd, Montenegro, while G3116 is a wild *T. monococcum* ssp. *boeoticum* from Lebanon. The F_{9-12} generations were prepared and phenotyped by Monika Škopová, Barbora Balcárková (Klocová, 2010), Lucia Hlusková (Gallová, 2011), and Hana Vanžurová (Vanžurová, 2013). The F_8 generation was provided by Prof. Jorge Dubcovsky, Ph.D., University of California (Dubcovsky et al., 1996). The mapping populations were grown in 2009–2012 (8 treatments in total; Table 1). Phenotyping methods are described in Vanžurová (2013) and summarized in Table 1.
  - Four 'verification' mapping populations created by a reciprocal cross of lines '144' and '246' (203 lines in total), a cross of DV92 and '113' (102 lines in total), and a cross of DV92 and '165' (102 lines in total). Lines 144, 246, 113, and 165 belong to the F_{12} mapping population. These populations were prepared by Barbora Balcárková and Hana Vanžurová. The plant phenotyping was done by Hana Vanžurová, Eva Malečková, Zuzana Korchanová, and Romana Nesnadná.
- A low path sequence of *T. monococcum* line TA4342-96 provided by Prof. Bikram S. Gill, Ph.D., Kansas State University, USA (personal communication, unpublished).
- Linkage map of *T. monococcum* DV92×G3116 constructed using the wheat 90K Infinium iSelect SNP array by Prof. Rudi Appels, Ph.D., Murdoch University, Australia (personal communication, unpublished).

Table 1. Traits and treatments in which they were evaluated.

Season	2009		2010	2011		2012		
Treatment	Stupice	Spring	Spring	Fall	Spring	Fall	Spring	Italy
Trait	2009	2009	2010	2010	2011	2011	2012	2012
Plant height	✓	✓	✓	✓	✓	✓	✓	✓
measured as the distance from the ground to the tip of the longest spike (excluding awns) at the milk ripening stage [cm]								
Number of tillers			✓	✓	✓	✓	✓	
measured as the number of culms 20 to 30 cm above the ground at the milk ripening stage								
Tillering pattern			✓	✓	✓	✓	✓	✓
visually evaluated and classified according to Bareš et al. (1985)								
Leaf pubescence	✓	✓	✓	✓	✓	✓	✓	✓
in 2009, the leaf pubescence was evaluated as the presence/absence of trichomes on the adaxial side of flag leaves; in 2010, the number of trichomes was counted at the beginning, middle and end of a flag leaf in areas 0.8 cm × leaf width; in 2011 and 2012, the number of trichomes was counted on a randomly selected vein (1 cm) in the middle part of a flag leaf								
Ear emergence time			✓	✓	✓	✓	✓	
calculated as days from planting to a glasshouse to the date when 50% of the spikes had emerged above the flag leaf collar								
Spike length		✓	✓	✓	✓	✓	✓	
the length of the five longest spikes (excluding awns) was measured at the milk ripening stage [cm]								
Number of spikelets per spike	✓	✓	✓	✓	✓	✓	✓	
the number of spikelets was counted on the five oldest spikes at the milk ripening stage								
Spike compactness		✓	✓	✓	✓	✓	✓	
calculated as the ratio of spike length to the number of spikelets								
Rachis brittleness		✓	✓	✓	✓	✓	✓	
the degree of shattering was determined on dried spikes by a comparison with their parents (G3116 has a brittle rachis, DV92 has a non-brittle rachis)								
Number of grains per spikelet			✓	✓	✓	✓	✓	✓
the grains were randomly dehusked until the number of both 1 in 1 and 2 in 1 grains reached at least the number of 50; the number of grains per spikelet was calculated as the ratio of the number of 1 in 1 grains to the total number of grains								
Grain weight (1 in 1)			✓	✓	✓	✓	✓	✓
Grain weight (2 in 1)			✓	✓	✓	✓	✓	✓
Grain weight (not differentiated)	✓	✓						
determined by weighing the 1 in 1 and 2 in 1 grains separately (they were not differentiated in 2009), transformed to a single 1 in 1 and 2 in 1 grain weight [g]								
Grain area (1 in 1)			✓	✓	✓	✓	✓	✓
Grain area (2 in 1)			✓	✓	✓	✓	✓	✓
Grain length (1 in 1)			✓	✓	✓	✓	✓	✓
Grain length (2 in 1)			✓	✓	✓	✓	✓	✓
Grain width (1 in 1)			✓	✓	✓	✓	✓	✓
Grain width (2 in 1)			✓	✓	✓	✓	✓	✓
measured on the MARVIN seed analyzer (see Chapter 4.4.1) [mm]								
Grain roundness (1 in 1)			✓	✓	✓	✓	✓	✓
Grain roundness (2 in 1)			✓	✓	✓	✓	✓	✓
calculated as the ratio of grain length to grain width								
Grain thickness (1 in 1)			✓	✓	✓	✓	✓	✓
Grain thickness (2 in 1)			✓	✓	✓	✓	✓	✓
calculated as the ratio of grain weight to grain area [g/mm^2]								
Grain protein content		✓	✓	✓	✓			
determined on the whole-grain dry matter by Fourier transform near-infrared (FT-NIR) spectroscopy								

Only some traits were assessed in each of the treatments as indicated by checkmarks; 1 in 1 – grains from spikelets that contained only one grain; 2 in 1 – grains from spikelets that contained two grains.

4.2 List of chemicals, kits, and solutions

4.2.1 Chemicals

- 3-methacryloxypropyltrimethoxysilane (Serva; Heidelberg, DEU)
- 40% acrylamide/bis-acrylamide solution 19:1 (5% crosslinker; Bio-Rad; Hercules, USA)
- agarose I (VWR; Radnor, USA)
- ammonium persulfate (APS; Sigma-Aldrich; St. Louis, USA)
- distilled water
- ethidium bromide (Sigma-Aldrich; St. Louis, USA)
- magnesium chloride, hexahydrate (Lach-Ner; Neratovice, CZE)
- N,N,N′,N′-tetramethylethylenediamine (TEMED; Bio-Rad; Hercules, USA)
- nucleotides; dATP, dCTP, dGTP, dTTP (VWR; Radnor, USA)
- primers (Eurofins Genomics; Luxembourg, LUX, and Integrated DNA Technologies; Coralville, USA)
- *Taq* polymerase (BioLabs; Ipswich, USA)

4.2.2 Kits

- Agencourt CleanSEQ (Beckman Coulter; Brea, USA)
- BDX64 Big Dye Enhancing Buffer (MCLAB; San Francisco, USA)
- BigDye Terminator 5× Sequencing Buffer (Applied Biosystems; Foster City, USA)
- BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; Foster City, USA)
- ExoSAP (Applied Biosystems; Foster City, USA)

4.2.3 Solutions

- **10× PCR buffer** (without Mg^{2+}):
    - 100 mM Tris-HCl, pH = 8,2 (Sigma-Aldrich; St. Louis, USA)
    - 500 mM potassium chloride (Lach-Ner; Neratovice, CZE)
    - 1% Triton X-100 (Sigma-Aldrich; St. Louis, USA)
- **10× PCR buffer** (with Mg^{2+}):
    - 100 mM Tris-HCl, pH = 8,2 (Sigma-Aldrich; St. Louis, USA)
    - 500 mM potassium chloride (Lach-Ner; Neratovice, CZE)
    - 15 mM magnesium chloride, hexahydrate (Lach-Ner; Neratovice, CZE)
    - 1% Triton X-100 (Sigma-Aldrich; St. Louis, USA)
- **100 bp DNA molecular weight marker** (520 µl):
    - 20 µl Gene Ruler 100 bp DNA Ladder Plus (Thermo Fisher Scientific; Waltham, USA)
    - 200 µl 6× STOP C DNA gel loading dye:
        - 100 mM EDTA (Sigma-Aldrich; St. Louis, USA)
        - 1% SDS (Sigma-Aldrich; St. Louis, USA)
        - 0,05% bromophenol blue (Sigma-Aldrich; St. Louis, USA)
        - 0,05% xylene cyanol (Sigma-Aldrich; St. Louis, USA)
        - 42,5% glycerol (Sigma-Aldrich; St. Louis, USA)
    - 300 µl distilled water
- **5× cresol red:**
    - 0,01% *o*-cresolsulfonephthalein (Sigma-Aldrich; St. Louis, USA)
    - 1,5% sucrose (Lach-Ner; Neratovice, CZE)
- **5× TBE buffer:**
    - 450 mM Tris (Sigma-Aldrich; St. Louis, USA)
    - 450 mM boric acid (Lach-Ner; Neratovice, CZE)
    - 10 mM EDTA, pH = 8,0 (Sigma-Aldrich; St. Louis, USA)

4.3 List of equipment

- automated liquid handler, Biomek NX^P (Beckman Coulter; Brea, USA)
- centrifuge, MiniStar Silverline (VWR; Radnor, USA)
- centrifuge, PlateFuge MicroCentrifuge (Benchmark Scientific; Edison, USA)
- horizontal electrophoresis system, Owl A6 (Thermo Fisher Scientific; Waltham, USA)
- laboratory scale, Vibra AJ-820CE (Shinko Denshi; Tokyo, JPN)
- microwave oven, KOR-6C2B (DAEWOO; Soul, KOR)
- optical grain analyzer, MARVIN (GTA Sensorik; Neubrandenburg, DEU)
- power supplies, MP-300V and MP-500V (Major Science; Saratoga, USA)
- Sanger sequencer, 3730xl DNA Analyzer (Applied Biosystems; Foster City, USA)
- thermocycler, C-1000 Touch (Bio-Rad; Hercules, USA)
- UV transilluminator, InGenius system (Syngene; Bengaluru, IND)
- vertical electrophoresis system, C-DASG-400-50 (C.B.S. Scientific; San Diego, USA
- vortex shaker, Reax Control (Heidolph Instruments; Schwabach, DEU)

4.4 Methods

4.4.1 Measurement of grain properties

For each line of the *T. monococcum* DV92×G3116 mapping population, randomly selected spikelets were trashed until both variants (1 in 1 = one grain per spikelet and 2 in 1 = two grains per spikelet) reached at least 50 seeds. Grains were measured using the MARVIN seed analyzer (GTA Sensorik; Neubrandenburg, DEU) and the average values of grain area (mm^2), grain length (mm), and grain width (mm) were obtained.

Then, the roundness of 1 in 1 and 2 in 1 grains was calculated as $\frac{\text{grain length}}{\text{grain width}}$, and grain thickness as $\frac{\text{grain weight}}{\text{grain area}}$ (g/mm^2).

4.4.2 Marker design

Markers were designed based on either SNP markers or annotated gene sequences.

Sequences of SNP markers were selected by manual integration of DArT markers into the *T. monococcum* SNP map (wheat 90K Infinium iSelect SNP map provided by Prof. Rudi Appels).

The annotated gene sequences were obtained by anchoring the chosen marker sequences to the syntenic regions of the cv. 'Chinese Spring' reference genome sequence (IWGSC RefSeq v1.0; IWGSC, 2018) using BLASTN (BLASTN v2.2.26; Altschul et al., 1997).

The sequences of SNP markers or the candidate gene sequences were searched in the database of *T. monococcum* low path sequence of line TA4342-96 using the BLASTN tool. The scaffolds producing significant alignments were checked for repetitive sequences using the non-redundant TREP (TRansposable Elements Platform) database (release 16; wheat.pw.usda.gov/cgi-bin/seqserve/blast_more.cgi).

FGENESH HMM-based gene structure prediction (Solovyev et al., 2006) with provided *Triticum aestivum* specific gene-finding parameters (softberry.com) was used to predict genes in the low copy regions. *In silico* translated proteins of the predicted genes were tested for uniqueness using BLASTP against the *Triticum* proteins (blast.ncbi.nlm.nih.gov/Blast.cgi). Genes for repetitive and common proteins (e.g., ribonuclease H, kinases, …) were discarded.

Primer pairs were designed using Primer3's default settings (v0.4.0; Untergasser et al., 2012) preferentially to conserved exon regions and their neighboring introns.

4.4.3 Polymerase chain reaction

PCRs were done in 96- or 384-well plates (VWR; Radnor, USA). Each PCR mixture (a total volume of 15 µL) consisted of 1× PCR buffer (without Mg^{2+}), 2 mM $MgCl_2$, 1× cresol red, 200 µM of each dNTP, 0.7 µM of each primer, 0.6 U of *Taq* polymerase and 15 ng of genomic DNA. Amplification was performed according to the touchdown PCR protocol presented in Table 2.

Table 2. Touchdown PCR protocol

	Step	Temperature [°C]	Duration	Cycles
1	initial denaturation	95 °C	5 min	1
2	denaturation	95 °C	40 sec	
3	annealing	65 °C (-0,7 °C/cycle)	40 sec	16
4	extension	72 °C	1 min	
5	denaturation	95 °C	40 sec	
6	annealing	53 °C	40 sec	17
7	extension	72 °C	1 min	
8	final extension	72 °C	10 min	1

4.4.4 Agarose gel electrophoresis

PCR products were separated by electrophoresis on 1.5% agarose gels in 0.5× TBE buffer at 130 V for 70 minutes and visualized by ethidium bromide staining.

4.4.5 Polyacrylamide gel electrophoresis

Alternatively, PCR products with small length polymorphism were separated by polyacrylamide gel electrophoresis in 0.5× TBE buffer at a constant 350 V for 90 minutes and visualized by ethidium bromide staining.

The 4% non-denaturing polyacrylamide gels consisted of 4% acrylamide/bis-acrylamide 19:1, 0.5× TBE buffer, 0.073% TEMED, 0.066% APS, and distilled water. The gels were run in 0.5× TBE buffer with 0.1‰ ethidium bromide.

4.4.6 Sanger sequencing

PCR products were purified by ExoSAP and then sequenced using Sanger sequencing method.

Firstly, the concentrations of PCR products were estimated by visually comparing the intensity of PCR products with bands of 200 ng/µl 100 bp DNA molecular weight marker (Gene Ruler; Thermo Fisher Scientific; Waltham, USA) separated by gel electrophoresis.

Then, 20–40 ng of PCR product, 0,25 U of thermosensitive alkaline phosphatase (FastAP; Applied Biosystems; Foster City, USA), 0,05 U of exonuclease I (Exo I; Applied

Biosystems; Foster City, USA), and 1× PCR buffer were incubated at 37 °C for 30 minutes and then denatured at 95 °C for 5 minutes.

The purified PCR products (5–20 ng) were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit. Each sequencing reaction (a total volume of 10 μL) contained 1× sequencing buffer, 0.875 μl of BDX64 (MCLAB; San Francisco, USA), 0.125 μl of BigDye (Applied Biosystems; Foster City, USA), and 1 M of primer. The cycle sequencing was performed in 96-well semi-skirted plates (Gel Company; San Francisco, USA) according to the following protocol (Table 3).

Table 3. Sequencing reaction protocol

Step	Temperature [°C]	Duration	Cycles
1	98	5 min	1
2	96	10 sec	
3	50	5 sec	60
4	60	4 min	

Finally, the products were purified on Biomek NX^P automated liquid handler (Beckman Coulter; Brea, USA) using CleanSEQ magnetic beads (Beckman Coulter; Brea, USA) and separated by capillary electrophoresis on ABI 3730xl DNA Analyzer (Applied Biosystems; Foster City, USA).

4.4.7 Genotyping

Presence-absence and length polymorphism markers were assessed by agarose and polyacrylamide gel electrophoresis. Marker genotypes were obtained by comparing the PCR profiles of parents with the profiles of their progeny.

PCR products of SNP markers were sequenced and electropherograms were aligned by MUSCLE algorithm (Edgar, 2004) in Geneious 6.1.8 (Biomatters Ltd.; Kearse et al., 2012). The genotypes were obtained by comparing the electropherograms of parents with their progeny.

Marker genotypes were typed into tables in Microsoft Excel (Microsoft Corp.). Experimental lines carrying the DV92 alleles were designated as A, and lines carrying the G3116 alleles were designated as B. Heterozygotes were designated as H.

4.4.8 Linkage map construction

Marker genotype data were loaded to Multipoint Ultradense (v4.1) mapping software (multiqtl.com; Ronin et al., 2017) and were processed as a RIL population with default settings. Markers were clustered into seven linkage groups (LGs) corresponding to seven *T. monococcum* chromosomes.

Local map stability and monotonicity of each LG were visually inspected and tested by jackknife resampling. Markers that were causing disarray were manually checked for segregation ratios, linkage distances, and marker associations and were eventually discarded to stabilize the map until the value of global variation decreased below 1.1.

LGs were assigned to chromosomes based on positions of known markers (according to Vanžurová, 2013) and then they were exported to Microsoft Excel with recombination frequencies converted into centiMorgans (cM) using the Kosambi mapping function (Kosambi, 1943). Exported LGs were visualized in MapChart 2.32 (Voorrips, 2002).

4.4.9 Quantitative trait loci analysis

Quantitative trait loci (QTL) analysis was performed by single-trait multiple environment multiple interval mapping (MIM) with marker restoration option in MultiQTL (v2.6) software (multiqtl.com; Korol et al., 2001). The skeleton linkage map was used for the analysis because skeleton markers are the most informative and less reliable attached markers caused disarray. Prior to the QTL analysis, trait values of each environment were normalized by dividing each trait value by standard deviation of the trait in Microsoft Excel.

LOD threshold values were determined by a global permutation test (1000 iterations). QTLs were declared significant when their LOD scores exceeded the 99% ($p < 0.01$) critical LOD threshold and highly significant when their LOD scores exceeded the 99.9% ($p < 0.001$) critical LOD threshold. Standard errors for the positions of the QTLs and 95% confidence intervals of the QTL spans were estimated using a bootstrap method (1000 iterations; Lebreton & Visscher, 1998). QTL effects were estimated as the percentage of explained variance (PEV) of the trait relative to its phenotypic variation. QTLs with PEV > 10% in at least half of the environments were considered to be major.

5 Results

5.1 Phenotyping of grain traits

T. monococcum is also called ‘einkorn’, which refers to the fact that the domesticated form has usually one grain per spikelet. The wild form, *T. boeoticum*, usually bears two grains per spikelet.

The experimental lines of the DV92×G3116 mapping population have either one or two grains per spikelet. The presence of two grains per one spikelet alters grain shapes compared to grains originating from spikelets with only one grain. For this reason, grains from spikelets with a different number of grains were analyzed separately.

For each line and each spikelet type from each treatment of the DV92×G3116 mapping population (except for the 2009 season), at least 50 grains (if available) were previously trashed. It was distinguished whether there was one (1 in 1) or two grains (2 in 1) per spikelet. An example of the difference in size between 1 in 1 and 2 in 1 grains can be illustrated on the parental lines DV92 and G3116 (Figure 3).

Figure 3. The differences in grain size and shape of the parental lines DV92 and G3116.

Grains from spikelets that contained just one grain are bigger and rounder compared to grains from spikelets with two grains. DV92 – cultivated *T. monococcum*; G3116 – wild *T. boeoticum*; 1 in 1 – grains from spikelets that contained just one grain; 2 in 1 – grains from spikelets that contained two grains. Scale bar = 1 cm.

Grain area (mm^2), grain length (mm), and grain width (mm) were measured on the MARVIN seed analyzer (GTA Sensorik; Neubrandenburg, DEU). Then, grain roundness ($\frac{\text{grain length}}{\text{grain width}}$) and grain thickness ($\frac{\text{grain weight}}{\text{grain area}}$; g/mm^2) were calculated for 1 in 1 and 2 in 1 grains separately.

Grain area and length are closely correlated. The longest grains (11.6 mm) also have the largest area (22.4 mm^2) and the shortest grains (3.9 mm) have the smallest area (7.6 mm^2). The most and least round grains have a similar length but a significantly different width (1.0 mm/4.6 mm). Thick grains are characterized by an increased weight, and thereafter volume, while thin grains are nearly flat.

5.2 Linkage map construction

A refined linkage map of *Triticum monococcum* DV92×G3116 included 699 markers composed of DArT (Jaccoud et al., 2001), SSR, STS, and IRAP markers, markers derived from BAC ends, and gene-based markers – previously used by Vanžurová (2013) – and another 17 markers designed in the present study (*Owm405*, *Owm406*, *Owm407*, *Owm411*, *Owm412*, *Owm413*, *Owm417*, *Owm419*, *Owm421*, *Owm423*, *Owm426*, *Owm429*, *Owm432*, *Owm433*, *Owm440*, *Owm441*, and *Owm445*; Appendix 6).

The resulting linkage map consisted of 676 markers (338 skeleton markers with 338 attached markers) and spanned 1033.1 cM on seven linkage groups, with one marker per 1.53 cM on average (Table 4).

5.3 QTL analysis

A total of 129 QTLs for 17 quantitative traits (Table 1) were mapped by multiple environment multiple interval mapping using the DV92×G3116 skeleton linkage map. QTLs are summarized in Appendix 2 and their location on chromosomes visualized in Appendix 1.

Out of all QTLs, 58 major QTLs are highly significant, 59 minor QTLs are highly significant, 1 major QTL is significant, and 11 minor QTLs are significant (Table 4).

The highest number of QTLs was mapped on $5A^m$ (22), while the lowest on $6A^m$ (10).

Table 4. Distribution of mapped markers and QTLs on seven *T. monococcum* chromosomes.

Chr.	Length [cM]	No. of markers	Average marker density [cM]	Highly sign. QTLs		Significant QTLs		Total no. of QTLs
				Minor	Major	Minor	Major	
$1A^m$	115.2	87	1.32	15	5		1	21
$2A^m$	158.7	100	1.59	4	16	1		21
$3A^m$	153.6	118	1.30	12	4	1		17
$4A^m$	73.8	52	1.42	6	8	3		17
$5A^m$	192.6	107	1.80	9	11	2		22
$6A^m$	164.8	74	2.23	8	1	1		10
$7A^m$	174.4	138	1.26	5	13	3		21
Total	1033.1	676	1.53	59	58	11	1	129

Highly significant QTLs – LOD scores exceeded the 99.9% ($p < 0.001$) LOD threshold; significant QTLs – LOD scores exceeded the 99% ($p < 0.01$) LOD threshold.

5.4 Marker saturation of regions containing the QTLs for leaf pubescence

Two QTLs for leaf pubescence were initially mapped on chromosomes $3A^m$ and $5A^m$ (Vanžurová, 2013) and had one of the highest LOD scores out of all QTLs. That is why we further focused on the characterization of the pubescence loci. Sequences of most of the wPt (DArT) markers developed for the *T. monococcum* mapping population are unavailable Therefore, linked markers (*wPt-470276*, *wPt-860735*, and *wPt-376043* on $3A^m$ and *wPt-470451*, *wPt-860900*, *wPt-862154*, and *wPt-470407* on $5A^m$) were integrated into the 90K iSelect SNP map constructed using 47 lines of our mapping population by comparing the genotypes and finding the best fitting positions in Microsoft Excel.

Then, 49 PCR markers (designated *Owm400–Owm448*) were designed to regions near the integrated markers on both chromosomes (*Owm400–Owm424* on $3A^m$ and *Owm425–Owm448* on $5A^m$) using available sequences of linked SNP markers (Appendix 6).

Markers *Owm405*, *Owm406*, *Owm407*, *Owm411*, *Owm412*, *Owm413*, *Owm417*, *Owm419*, *Owm421*, *Owm423*, *Owm426*, *Owm429*, *Owm432*, *Owm433*, *Owm440*, *Owm441*, and *Owm445* were polymorphic in the *Triticum monococcum* DV92×G3116 F_{12} mapping population and were added to the linkage map (Chapter 5.1).

Markers *Owm407*, *Owm412*, *Owm419*, *Owm421*, *Owm423*, *Owm426*, *Owm432*, *Owm433*, *Owm440*, *Owm441*, and *Owm445* were also found to be polymorphic on the F_2 verification mapping populations (144×246, 246×144, DV92×113, and DV92×165).

The final QTL analysis confirmed that the QTLs for leaf pubescence map close to the markers ***wPt-860735*** ($3A^m$) and ***wPt-470407*** ($5A^m$, allelic to *wPt-376529*; Appendix 3).

The closest markers with known sequences were anchored to the reference sequence of *Triticum aestivum* cv. 'Chinese Spring' (IWGSC RefSeq v1.0; IWGSC, 2018).

Alignments located the **3A^m markers** *Owm405*, *Owm407*, *Owm412*, *Owm417*, *Owm421*, *Owm423*, *wPt-9638*, and *D_contig33074_133* (SNP marker near *wPt-470276* from the DV92×G3116 SNP linkage map) in the physical region of ~30 Mbp on chromosome 3A, ~42 Mbp region on 3B, and ~28 Mbp region on 3D, with the most significant alignments being on 3B. According to the constructed linkage map, marker *wPt-860735* was located between markers *wPt-470276* and *Owm405* (Appendix 3), so a new set of markers designated *Owm449–Owm466* was designed in the region between 660 and 690 Mbp on 3B (Appendix 7).

In a similar way to the 3A^m markers, the **5A^m markers** *Owm426*, *Owm433*, *Owm440*, *Owm441*, *Owm445*, *wPt-862154*, *wPt-470407* (*wPt-376529*), and *wPt-469600* were located in the region of ~34 Mbp on chromosome 5A. Marker *wPt-470407* (*wPt-376529*) was located between markers *Owm445* and *wPt-469600* (Appendix 3), and new markers designated *Owm467–Owm484* were designed in the region between 688 and 691 Mbp on 5A (Appendix 7).

Markers *Owm453*, *Owm457*, *Owm462*, *Owm463*, *Owm466*, *Owm467*, *Owm471*, *Owm476*, *Owm478*, *Owm480*, and *Owm483* were polymorphic in the *T. monococcum* DV92×G3116 F_{12} mapping population and were integrated into the final DV92×G3116 linkage map. The marker loci order in the regions with the newly added markers on 3A^m and 5A^m was adjusted (Appendix 4). Then, a leaf pubescence QTL analysis was done using the global linkage map (with both skeleton and attached markers) and refined QTL positions were obtained.

The QTL for leaf pubescence on **3A^m** was mapped within a marker interval between *Owm412* and *Owm421* (~709–729 Mbp region on 3B, IWGSC RefSeq v1.0; IWGSC, 2018) near markers *wPt-860851* and *wPt-9638* (Appendix 4).

The QTL for leaf pubescence on **5A^m** was mapped within a marker interval between *Owm471* and *Owm476* (~688.7–689.9 Mbp region on 5A, IWGSC RefSeq v1.0; IWGSC, 2018) between markers *wPt-470407* (*wPt-376529*) and *wPt-469153* (*wPt-469591*; Appendix 4).

The positions of QTLs on 3A^m and 5A^m were verified on the verification mapping populations by single environment multiple interval mapping. The QTL on 3A^m was verified within the marker interval between *Owm412* and *Owm421* and the QTL on 5A^m was verified near *wPt-862154* (Appendix 5).

6 Discussion

Triticum monococcum (or einkorn wheat) is an ancient small grain cereal that has played a major role in the origins of Neolithic agriculture and its expansion beyond the area of Fertile Crescent. Einkorn is a good model for wheat genomic studies because of its diploid genome and good collinearity with other *Triticum* species (Dubcovsky et al., 1996; Marino et al., 2018; Yu et al., 2019).

Previously constructed *T. monococcum* linkage maps showed varying lengths (Table 5). The length of the linkage map constructed in the present study (Appendix 1) is comparable to them considering the type and number of markers used.

Table 5. A comparison of various *T. monococcum* linkage maps.

Reference	Pop.	Cross	Length [cM]	No. of markers	Type of markers
Dubcovsky et al. (1996)	F_2	DV92×G3116	1067	335	RFLP, isozymes, seed storage-protein markers, morphological trait loci
Kojima et al. (1998)	F_3	KT3-5×KT1-1	862	81	RFLP, RAPD, ISSR
Taenzler et al. (2002)	RIL	ID69×ID49, ID362×ID1331	856	477	RFLP, AFLP, morphological trait loci, seed storage-protein markers
Singh et al. (2007)	RIL	pau14087×pau5088	1262	176	RFLP, SSR, EST
Hori et al. (2007)	RIL	KT3-5×KT1-1	1038	341	RFLP, EST
Jing et al. (2009)	F_2	MDR308×MDR002	1063	356	DArT, SSR, morphological trait loci
Yu et al. (2016)	RIL	KT3-5×KT1-1	1377	926	DArT, SSR, gene markers, seed storage-protein markers
Marino et al. (2018)	F_2	ID228×ID752, ID396×ID752	1562	2988	DArT-seq
Yu et al. (2019)	RIL	KT3-5×KT1-1	1873	9937	RAD-seq, DArT, SSR
The present study	RIL	DV92×G3116	1033	716	DArT, SSR, STS, IRAP, markers derived from BAC ends, gene markers

Pop. – type of population that was used for linkage map construction.

Accessions:

DV92 (MDR308), KT3-5, ID69, ID362, ID396, ID1331, MDR002, and pau14087 – cultivated *T. monococcum*;

G3116, KT1-1, ID49, ID752, and pau5088 – wild *T. boeoticum*;

ID228 – feral *T. monococcum* ssp. *aegilopoides* sensu Schiemann

RFLP – restriction fragment length polymorphism; RAPD – random amplified polymorphic DNA; ISSR – inter-simple sequence repeat; AFLP – amplified fragment length polymorphism; SSR – simple sequence repeats; EST – expressed sequence tag; DArT – Diversity Arrays Technology; RAD – restriction site-associated DNA; STS – sequence-tagged site; IRAP – inter-retrotransposon amplified polymorphism; BAC – bacterial artificial chromosome.

A multiple environment multiple interval QTL mapping analysis of our mapping population revealed 129 QTLs for 17 quantitative traits (Appendix 1 and 2).

Most of the QTLs for plant height, tillering pattern, leaf pubescence, ear emergence time, spike length, the number of spikelets per spike, rachis brittleness, the number of grains per spikelet, grain weight, and grain protein content mapped by Vanžurová (2013) were also detected in the present study. However, some of them were identified in different positions.

The differences may be caused by the employment of different statistical methods. Vanžurová (2013) used single environment simple interval mapping, while multiple environment multiple interval mapping was used in the present study.

In our case, the QTL contribution (an average percentage of variance explained by QTLs; or PEV) ranged from 31% (the number of grains per spikelet) to 72% (leaf pubescence). Therefore, a significant portion of the PEV remains undetected. There are several possible reasons why this is the case, e.g., (a) minor QTLs that have not been detected above LOD threshold, (b) environmental (or residual) variance that can arise from both environmental factors and genetic background, and (c) epistatic interactions.

Most of the QTLs were mapped in chromosomal regions similar to those in which QTLs were previously reported. A comparison of QTL positions mapped in the present study between QTLs mapped in other studies is problematic as different mapping populations grown in various environments and different types of markers are used. Therefore, approximate relative QTL positions (a ratio of the QTL position to a total length of a chromosome) were compared.

6.1 Plant height

Plant height directly affects yield because tall plants easily lodge, which causes yield loss. However, if the plants are too short, ventilation and light are limited in the lower part due to the close packing, which reduces the photosynthetic efficiency and yield. Thus, the optimal height is essential for a high yield. Plant height is influenced by both Mendelian and quantitative genes. Two major QTLs for plant height were mapped on $2A^m$ and $7A^m$, and five minor QTLs on chromosomes $1A^m$, $3A^m$, $4A^m$, $5A^m$, and $6A^m$.

The QTLs mapped on **$1A^m$**, **$3A^m$**, **$5A^m$**, **$6A^m$**, and **$7A^m$** are in similar positions as QTLs mapped in diploid wheat on $5A^m$L (Hori et al., 2007) and in hexaploid wheat on 1BL (Lu et al.; 2012), 3A and 3D (Liu et al., 2014; Zhang et al., 2017b; Zhou et al., 2017; Zhao et al., 2019), 5AL and 5BL (Lyra et al., 2020), 6AL and 6BL (Würschum et al., 2017; Zhang et al., 2017b; Zhao et al., 2019), and 7A and 7B (Huang et al., 2004; Liu et al., 2014; Gao et al., 2015). According to Würschum et al. (2017), the identified QTL on 6AL might be the *Reduced height* gene *Rht24* (Tian et al., 2017).

The QTL on **$2A^m$** has the highest PEV (21%) among the QTLs for plant height and was mapped in the same region as QTLs mapped in *T. monococcum* (Hori et al., 2007; Yu et al., 2016) and QTLs mapped on 2A, 2B, and 2D in hexaploid wheat (Liu et al., 2014; Gao et al., 2015; Würschum et al., 2017). Yu et al. (2016) suppose that the QTL might be homoeologous to *Rht8* (*Reduced height 8*) in *T. aestivum* (Korzun et al., 1998; Gasperini et al., 2012).

Reduced height dwarfing genes *Rht-B1* and *Rht-D1* (Peng et al., 1999) are located on chromosomes 4B and 4D. Their exploitation in breeding is one of the reasons why the ‘Green

Revolution' was so successful (Borlaug, 1971). The QTL mapped on **4A^{m}** appears to be in a similar region and it might be homoeologous to *Rht-1*. Furthermore, Yu et al. (2016) mapped a QTL for plant height on 4A^{m} in the same position and confirmed by markers that *Rht-1* is most likely an underlying gene.

6.2 The number of tillers and tillering pattern

The number of tillers is an agronomically important trait because it directly affects the number of spikes per plant, a key component of grain yield, and it also determines plant canopy size and photosynthetic area. High tillering is not desired as it leads to yield reduction because tillers drain nutrients from the main shoot but undergo senescence before achieving maturity (Kebrom et al., 2012). Furthermore, free-tillering cultivars are less productive compared with low tillering ones under drought conditions (Richards, 1988). Two major QTLs for the number of tillers were mapped on 1A^{m} and 7A^{m} (PEV = 9% and 10%, respectively), and two minor QTLs on 2A^{m} and 4A^{m}.

The QTLs mapped on **2A^{m}**, **4A^{m}**, and **7A^{m}** are in similar positions as QTLs reported on 2B and 2D (Li et al., 2002; Wang et al., 2016a; Xu et al., 2016), 4DS (Ren et al., 2018), and 7A (Huang et al., 2004) in *T. aestivum*.

Tiller inhibition 1 (*tin1*) gene was mapped on 1AS (Richards, 1988; Spielmeyer et al., 2004); however, the QTL mapped in the present study is on **1A^{m}L**. A QTL was mapped in a similar region on 1BL in hexaploid wheat by Liu et al. (2018a).

No QTL was mapped on 3A^{m} where *tin3*, a recessive gene that inhibits tillering, was identified in *T. monococcum* by Kuraparthy et al. (2006). The mapping population used by Kuraparthy et al. (2006) was prepared using mutagenesis and selected for *tin3* mutants; therefore, it was not expected that *tin3* would manifest in our mapping population.

A sufficient number of tillers and their shape is one of the characteristics responsible for weed competitiveness and reduced soil water evaporation. Ground cover at early tillering is strongly correlated with weed suppression throughout the season (Kruepl et al., 2006). However, plants with prostrate shape are susceptible to diseases (such as powdery mildew, *Blumeria graminis* (DC.) Speer); therefore, drooping or loosely spreading **tillering pattern** (also plant shape or architecture) might be the most optimal. A major QTL for tillering pattern was mapped on 2A^{m} (PEV = 21%), and four minor QTLs were mapped on 1A^{m}, 4A^{m}, 5A^{m}, and 7A^{m}. The QTLs for the number and shape of tillers on **1A^{m}**, **2A^{m}**, and **7A^{m}** mapped in the same region; thus, the underlying genes might be controlling both the number and pattern of tillers. That is in agreement with Li et al. (2002) who mapped a QTL for the number of tillers in a similar region on 2D in *T. aestivum*, which also influenced tillering pattern. Yu et al. (2016) mapped QTLs for plant architecture on 2A^{m} and 7A^{m} in syntenic positions.

Both traits appear to be heavily influenced by the environment as the QTLs for the number of tillers and tillering pattern explained only 49% and 33% of the total variance, respectively.

6.3 Leaf pubescence

Leaf pubescence (or hairiness) provides a protection against both biotic and abiotic stress factors. It might be an important target in breeding drought-resistant cultivars. A major QTL for leaf pubescence (PEV = 57%) was mapped on $3A^m$, and two minor QTLs were mapped on $5A^m$ (PEV = 13%) and $7A^m$ (PEV = 2%). The QTLs on both $3A^m$ and $5A^m$ were selected for verification and were saturated with markers as one of the most statistically significant QTLs detected in our population (Appendix 2).

A QTL for leaf pubescence has not been previously mapped on chromosome 3 of wheat.

The QTL on **$5A^m$** was mapped in a syntenic position in diploid wheat by Hori et al. (2007), Jing et al. (2007, 2009), and Yu et al. (2016).

The QTL on **$7A^m$** has not been previously mapped in diploid wheat but *Hl2* (*leaf hairiness 2*) and two other QTLs were detected in *T. aestivum* on 7B, and 7A and 7D, respectively (Taketa et al., 2002, Doroshkov et al., 2015). However, this QTL on $7A^m$ was not further pursued as its effects are minor (PEV = 2%) and is statistically less significant compared to the highly significant QTLs on $3A^m$ and $5A^m$ (Appendix 2).

The QTL on $3A^m$ was located within a marker interval between *Owm412* and *Owm421* corresponding to a physical interval of ~20 Mbp on 3B (~709–729 Mbp; IWGSC RefSeq v1.0; IWGSC, 2018), which was narrowed down from the initial ~42 Mbp interval. Nevertheless, the ~20 Mbp interval still represents a large part of the chromosome and contains 84 predicted genes (high-confidence genes, IWGSC RefSeq v1.0; IWGSC, 2018). The QTL appears to be near the centromeric region, which might be the reason why it is harder to narrow it down as the amount of recombination is limited.

The QTL on $5A^m$ was located within a marker interval between *Owm471* and *Owm476* corresponding to a physical interval of ~1.2 Mbp on 5A (~688.7–689.9 Mbp; IWGSC RefSeq v1.0; IWGSC, 2018), narrowed down from the initial ~34 Mbp interval. The ~1.2 Mbp interval contains 26 predicted genes (high-confidence genes, IWGSC RefSeq v1.0; IWGSC, 2018).

6.4 Ear emergence time

Regional and seasonal adaptation of wheat varieties largely depends on ear (spike) emergence time. Appropriate ear emergence time and anthesis are important targets for breeding. They correlate with the growth period and affect yield. Two major QTLs for ear emergence time were mapped on $1A^m$ and $7A^m$, and four minor QTLs on $2A^m$, $4A^m$, $5A^m$, and $6A^m$.

The QTL on **$1A^m$** (PEV = 33%) mapped in the same position as the previously reported (Bullrich et al., 2002; Valárik et al., 2006) wheat ortholog of circadian clock regulator *EARLY FLOWERING 3* (*ELF3*; Alvarez et al., 2016), formerly named earliness *per se* gene *Eps-A^m1*. In hexaploid wheat, three QTLs were mapped in similar regions on 1AL (Kuchel et al., 2006), 1DL (Griffiths et al., 2009), and 1BL (Zikhali et al., 2017).

Kuchel et al. (2006) and Bennett et al. (2011) mapped homoeologous QTLs for ear emergence time on 2AS, 2BS, and 2DS (coincident with the diagnostic photoperiod responsive allele *Ppd-D1a* marker; Beales et al., 2007) in a similar region to that on **$2A^m$** from the present study. Moreover, the QTLs for tiller number and spike length on $2A^m$ mapped in a similar position, which is in agreement with reports (Li et al., 2002; Xie et al., 2015; Ochagavía et al., 2017) that *Ppd-1* alleles affect tillering and spike-related traits (Boden et al., 2015).

The *VRN1* vernalization genes (Yan et al., 2003) are located on $5A^m$L, 5AL, 5BL, and 5DL (Dubcovsky et al., 1998; Barrett et al., 2002) and the *VRN2* gene (Yan et al., 2004) was mapped in the distal region of $5A^m$L (Dubcovsky et al., 1998). Yu et al. (2016) used *VRN* specific STS markers and mapped *VRN1* in the middle of the long arm of chromosome $5A^m$ and *VRN2* in the distal region of $5A^m$. In the present study, the QTL on **$5A^m$** was mapped in the distal region as well; thus, it is most likely the *VRN2* gene.

The QTL mapped on **$7A^m$** (PEV = 16%; marker *FT*, 63.1 cM) is the *TaFT-VRN3* flowering gene (Yan et al., 2006) mapped on 7BS (Huang et al., 2003; Yan et al., 2006; Griffiths et al., 2009) and $7A^m$S (Yu et al., 2016).

The QTLs on **$4A^m$** and **$6A^m$** were mapped in regions similar to those with QTLs on 4AS and 4BS (Bennett et al., 2011; Chen et al., 2020), and 6AL and 6BL (Börner et al., 2002; Huang et al., 2003; Griffiths et al., 2009; Chen et al., 2020).

6.5 Spike-related traits

Most spike-related traits, such as spike length, the number of spikelets per spike, and spike density, positively affect the number of grains per spike, which in turn affects the yield (Liu et al., 2018b).

In *T. aestivum*, four well-studied genes affect spike-related traits: *Q*, *Br*, *C*, and *s*. The domestication *Q* gene on chromosome 5AL (Simons et al., 2006) pleiotropically affects a repertoire of traits, e.g., spike length and shape, seed threshability, glume tenacity, rachis

fragility, plant height, and ear emergence time. *T. monococcum* has the primitive *q* allele, which grants the non-free-threshing character.

In addition to the *Q* gene, *Brittle rachis* (*Br*) loci on the homoeologous group 3 chromosomes control the rachis character (Chen et al., 1999; Watanabe & Ikebata, 2000; Watanabe et al., 2003; Li & Gill, 2006; Nalam et al., 2006).

The *C* gene on 2D (Johnson et al., 2007) affects spike morphology, grain size, shape, and number, and is characteristic for *T. aestivum* ssp. *compactum* (Host) Mac Key (or club wheat), which has characteristic compact spikes.

The *s* gene on 3DS (Salina et al., 2000) is characteristic for another subspecies, *T. aestivum* ssp. *sphaerococcum* (Percival) Mac Key (or shot wheat), and determines whether a spike is short and dense and has round grains and glumes. According to Faris et al. (2014), these genes do not contribute to various spike-related traits among the modern *T. aestivum* cultivars as all of them have the universal *QcS* genotype.

Two major QTLs for **spike (ear) length** were mapped on $2A^m$ and $4A^m$, and four minor QTLs were mapped on $1A^m$, $3A^m$, $5A^m$, and $6A^m$.

The QTLs are in similar positions as QTLs mapped in diploid wheat on $4A^m$ and $5A^m$ (Hori et al., 2007; Yu et al., 2016) and in hexaploid wheat on 1B and 1D (Börner et al., 2002; Marza et al., 2005; Kumar et al., 2006), 2B and 2D (Börner et al., 2002; Kumar et al., 2006; Cui et al., 2011; Liu et al., 2018b), 3B (Li et al., 2015; Würschum et al., 2018; Zhou et al., 2017), 4A and 4B (Börner et al., 2002; Chu et al., 2008; Cui et al., 2011; Faris et al., 2014; Gao et al., 2015; Li et al., 2015), 5A, 5B, and 5D (Kato et al., 1999; Börner et al., 2002; Cui et al., 2011; Zhai et al., 2016; Zhou et al., 2017), and 6A (Börner et al., 2002; Liu et al., 2018b; Würschum et al., 2018).

The number of spikelets per spike has a significant effect on the number of grains per spike and grain weight. Three major QTLs for spikelet number per spike were mapped on $1A^m$, $2A^m$, and $7A^m$, and two minor QTLs in wide intervals on $5A^m$ and $6A^m$.

The QTL on **$1A^m$** was mapped in the same position as *ELF3* (marker *SMP*; 115.2 cM), which have a role in the regulation of flowering and the number of spikelets per spike. *ELF3* shortens the vegetative and the spike initiation phase; therefore, fewer spikelets and grains per spike are produced (Lewis et al., 2008).

The QTLs mapped on **$2A^m$**, **$5A^m$**, and **$6A^m$** are in similar positions as QTLs mapped in hexaploid wheat on 2BS and 2DS (Cui et al., 2011; Zhang et al., 2018; Ma et al., 2019a; Kuzay et al., 2019), 5A and 5B, and 6A and 6D (Wang et al., 2010; Cui et al., 2011; Zhang et al., 2018).

Hori et al. (2007) mapped QTLs for the number of spikelets per spike on $3A^m$ and $4A^m$ in diploid wheat but they were not detected in the present study.

Xu et al. (2013), Zhang et al. (2018), Fan et al. (2019), Kuzay et al. (2019), and Chen et al. (2020) mapped QTLs on 7AS and 7AL and Yu et al. (2016) mapped a QTL on 7A^{m}S. Interestingly, the QTL on **7A^{m}** mapped in the present study consists of two peaks (one of them on 7A^{m}S and more distinct one on 7A^{m}L) that approximately correspond to the QTLs on 7AS, 7A^{m}S, and 7AL. A QTL on 7AL has been recently discovered to be an ortholog of rice *ABERRANT PANICLE ORGANIZATION 1* (*TaAPO-A1*; Muqaddasi et al., 2019).

Spike compactness (density) is associated with grain yield. It is determined as the ratio of the number of spikelets per spike to spike length and it is positively correlated with the number of spikelets per spike and negatively correlated with spike length (Würschum et al., 2018). Varieties with longer and more compact spikes bear a higher number of grains per spike; therefore, breeding for length and density of spikes can improve grain yield (Li et al., 2015). Low spike density plants have a lower number of spikelets on spikes, and, therefore, reduced yield, but the spikes have better ventilation. On the contrary, high-density spikes tend to hold more water; thus, pre-harvest sprouting is more likely to occur, which leads to yield and quality reduction and higher incidence of Fusarium head blight (Mesterházy, 1995). Therefore, varieties with a moderate spike density are preferred (Li et al., 2015). Two major QTLs for spike density were mapped on 3A^{m} and 4A^{m}, and five minor QTLs on 1A^{m}, 2A^{m}, 5A^{m}, 6A^{m}, and 7A^{m}.

The QTLs were mapped in chromosomal regions similar to those with QTLs detected in *T. aestivum* on 1B (Liu et al., 2019), 2A, 2B, 2D (Sourdille et al., 2003; Cui et al., 2011; Faris et al., 2014; Echeverry-Solarte et al., 2015; Li et al., 2015; Zhou et al., 2017; Würschum et al., 2018), 3A, 3B (Cui et al., 2011; Faris et al., 2014; Liu et al., 2019), 4A, 4B (Katkout et al., 2014; Liu et al., 2019), 5A, 5B, 5D (Chu et al., 2008; Katkout et al., 2014; Echeverry-Solarte et al., 2015; Zhai et al., 2016; Liu et al., 2019), 6A (Cui et al., 2011; Würschum et al., 2018; Liu et al., 2019), and 7A (Würschum et al., 2018).

The QTL on **2A^{m}** was mapped in the same position as the QTL for plant height, which might be homoeologous to *Rht8* as discussed above. This is in agreement with Liu et al. (2019) who suggest that spike density might be affected by dwarfing genes.

Rachis brittleness (also shattering or fragility) is characteristic for wild *T. boeoticum* that the spikelets disarticulate at maturity to disperse the seed. Domesticated *T. monococcum* has a non-brittle rachis that breaks into spikelets only during threshing or flailing. The loss of a brittle rachis was one of the first and most important domestication traits acquired by the cultivated wheats. The non-brittle rachis einkorn originated from a single nucleotide mutation at *Btr1* on 3A^{m}S (Pourkheirandish et al., 2018). This mutation was selected as a result of cultivation by early Neolithic farmers. The trait is influenced by QTLs other than the *btr1* but with milder phenotypic effects (Jiang et al., 2014). Two major QTLs for rachis fragility were mapped on 3A^{m} and 7A^{m}, and three minor QTLs on 1A^{m}, 4A^{m}, and 5A^{m}.

The QTLs on **1A^m** and **4A^m** have not been previously mapped. The QTLs on **5A^m** and **7A^m** were mapped in chromosomal regions similar to those with QTLs detected in *T. aestivum* on 5A and 7B (Marza et al., 2005).

The major QTL on 3A^m was mapped in a similar position as *Btr1* mapped in *T. monococcum* by Pourkheirandish et al. (2018).

The number of grains per spikelet determines the number of grains per spike and thus determines the yield potential. In general, domesticated *T. monococcum* has one grain per spikelet, whereas wild *T. boeoticum* has two grains per spikelet. Current *T. aestivum* cultivars produce three to five grains per spikelet. Two major QTLs for the number of grains per spikelet were mapped on 1A^m and 3A^m, and one minor QTL on 5A^m. Only 31% of variance was explained by the QTLs, which indicates that the grain number might be strongly influenced by the environment.

Singh et al. (2008) mapped a QTL for the number of grains per spikelet on 1A^{m}L in *T. monococcum* at a different position than the QTL mapped on **1A^m** in the present study. Singh et al. (2008) also mapped a QTL on 4A^{m}S in *T. monococcum* but no QTL was detected on 4A^m in the present study. A QTL in a similar region as the QTL mapped on 1A^m was detected in *T. aestivum* on 1D by Zhou et al. (2017).

Recently, the *Grain Number Increase 1* (*GNI1*) gene was identified on 2AL in diploid and polyploid wheat (Sakuma et al., 2019). However, no QTL on 2A^m was detected in the present study.

The QTL on **3A^m** was mapped in a region similar to those with QTLs mapped in *T. aestivum* on 3A, 3B, and 3D (Guo et al., 2016; Zhou et al., 2017)

6.6 Grain-related traits

One of the main components of yield is **grain weight**, which is largely defined by grain size and the morphometric characteristics of grain area, length and width.

In *T. aestivum*, many QTLs associated with grain weight have been found across the wheat genome on most of the chromosomes (e.g., Börner et al., 2002; Groos et al., 2003; Huang et al., 2004; McCartney et al., 2005; Quarrie et al., 2005; Marza et al., 2005; Narasimhamoorthy et al., 2006; Kuchel et al., 2006; Wang et al., 2009; Zheng et al., 2010; Reif et al., 2011; Wu et al., 2012; Lopes et al., 2013; Zhang et al., 2013; Zanke et al., 2015; Kumar et al., 2016; Zhang et al., 2016; Assanga et al., 2017; Deng et al., 2017; Cabral et al., 2018; Su et al., 2018; Würschum et al., 2018; Zhang et al., 2018; Goel et al., 2019; Kumari et al., 2019; Ma et al., 2019b; Wang et al., 2019; Xin et al., 2020). Nevertheless, only few genes were characterized (see Li & Yang, 2017), e.g., *TaGW2* (Su et al., 2011; Hong et al., 2014), *TaGS5* (Wang et al., 2016b), and *TaGW8* (Yan et al., 2019).

In the present study, the weight of 1 in 1 and 2 in 1 grains (one grain per spikelet or two grains per spikelet) was analyzed separately (except for the 2009 season in which the 1 in 1 and 2 in 1 grains were not differentiated). This allowed more precise QTL mapping as 1 in 1 grains were 19% heavier compared to 2 in 1 grains. This is also supported by the fact that the QTLs for the weight of non-differentiated grains were detected in wider intervals. Moreover, a higher number of QTLs was detected for the weight of 1 in 1 and 2 in 1 grains. No other studies have analyzed 1 in 1 and 2 in 1 grains separately.

Grains of the wild *T. boeoticum* parent were found to be weighing 40% less compared to grains of the domesticated *T. monococcum* parent. This is similar to a 60% weight difference reported by Yu et al. (2019). The average grain weight of RILs (18.4 mg) was 1.8 mg higher than the grain weight of the KT1-1×KT3-5 RIL population used by Yu et al. (2019). The grains of the KT1-1 and KT3-5 parental lines were 22% lighter than grains of the DV92 and G3116 parents, so the difference was expected.

In general, three major QTLs for grain weight were mapped on $2A^m$, $5A^m$, and $7A^m$, and three minor QTLs on $1A^m$, $3A^m$, and $4A^m$. The QTLs for the weight of 1 in 1, 2 in 1, and non-differentiated grains mapped in similar regions; therefore, the underlying genes are most likely the same. The QTLs on $2A^m$ are an interesting exception because peaks of the QTLs for the weight of 1 in 1 and 2 in 1 grains mapped 13.4 cM apart. There is a similar difference in the position of peaks of QTLs for the area, length, and width of 1 in 1 and 2 in 1 grains on $2A^m$. Thus, different genes on $2A^m$ might be affecting the development of 1 in 1 and 2 in 1 grains.

The QTLs mapped on **$2A^m$** and **$7A^m$** are in similar positions as QTLs mapped in *T. monococcum* (Hori et al., 2007; Singh et al., 2008). Yu et al. (2019) mapped QTLs for grain weight on $1A^m$, $2A^m$, $3A^m$, $5A^m$, and $7A^m$. The QTLs on **$1A^m$**, **$3A^m$**, and **$5A^m$** are in similar regions, while the QTLs on $2A^m$ and $7A^m$ are not.

Grain size (grain area, length, and width) and shape (grain roundness, thickness) are important characteristics that are components of yield and milling quality and have a strong positive correlation with grain weight (Gegas et al., 2010). The transition from wild wheats to their domesticated forms was associated with a trend toward larger grains. The shape of grains has changed from long and thin (cylindrical) into wider and shorter (Fuller, 2007). However, the grain shape has not been a major component of the wheat domestication syndrome but has become an important breeding target due to the market and industry requirements. In general, large thin-skinned grains with nearly circular shape have higher flour extraction rates (Gegas et al., 2010).

In *T. aestivum*, QTLs for grain shape and size have been detected on almost all wheat chromosomes (e.g., Gegas et al., 2010; Tsilo et al., 2010; Prashant et al., 2012; Maphosa et al., 2014; Williams & Sorrells, 2014; Wu et al., 2015; Kumar et al., 2016; Brinton et al., 2017; Su

et al., 2018; Kumari et al., 2019; Ma et al., 2019b; Wang et al., 2019; Xin et al., 2020). In *T. monococcum*, Yu et al. (2019) have detected QTLs on all chromosomes except $4A^m$.

The average values of area (mm^2), length (mm), and width (mm) of 1 in 1 (15.5, 7.7, and 2.7, respectively) and 2 in 1 grains (12.5, 7.5, and 2.1) were similar to those reported by Yu et al. (2019; 12.9, 7.8, and 2.2). The grains of the KT1-1×KT3-5 RIL population used by Yu et al. (2019) were 8% smaller overall, but slightly more elongated.

Three major QTLs for the **area** of both 1 in 1 and 2 in 1 grains were mapped on $2A^m$, $5A^m$, and $7A^m$, and two minor QTLs on $1A^m$ and $3A^m$. The QTLs on $1A^m$ and $5A^m$ overlap, which means that the underlying genes are probably the same, while the QTLs on $2A^m$, $3A^m$, and $7A^m$ mapped in different chromosomal regions. The QTLs on $3A^m$ mapped 60 cM apart; however, they both consist of two peaks. One of the peaks is in the telomeric region (above the LOD threshold in the case of 2 in 1 grains), while the second one is in the centromeric region (above the LOD threshold in the case of 1 in 1 grains). Therefore, there might be two different QTLs for grain area on $3A^m$. The QTLs on **$3A^m$** (in the telomeric region) and **$5A^m$** mapped in similar positions as the QTLs detected by Yu et al. (2019). The QTL on $5A^m$ explained up to 26% of variance, which is similar to what Yu et al. (2019) reported (22%).

Two major QTLs for the **length** of both 1 in 1 and 2 in 1 grains were mapped on $2A^m$, and $7A^m$, one major QTL for the length of 2 in 1 grains on $5A^m$, three minor QTLs for the length of both 1 in 1 and 2 in 1 grains on $1A^m$, $3A^m$, $4A^m$, and one minor QTL for the length of 1 in 1 grains on $5A^m$. The QTLs on $3A^m$, $4A^m$, $5A^m$, and $7A^m$ overlap and the QTLs on $2A^m$ are in a similar position. No QTLs were mapped in positions similar to those in which Yu et al. (2019) detected QTLs for grain length.

Three major QTLs for the **width** of both 1 in 1 and 2 in 1 grains were mapped on $2A^m$, $4A^m$, and $5A^m$, one major QTL for the length of 1 in 1 grains on $7A^m$, one major QTL for the length of 2 in 1 grains on $1A^m$, one minor QTL for the length of both 1 in 1 and 2 in 1 grains on $3A^m$, and three minor QTLs for the length of either 1 in 1 or 2 in 1 grains on $1A^m$, $6A^m$, and $7A^m$. The QTLs $2A^m$, $4A^m$, $5A^m$, and $7A^m$ overlap. The QTLs on **$1A^m$** for the width of 1 in 1 grains and on **$5A^m$** for the width of both 1 in 1 and 2 in 1 grains were mapped in positions similar to those with QTLs detected by Yu et al. (2019).

The ratio of grain length to grain width was designated as **grain roundness**. One major QTL for the roundness of both 1 in 1 and 2 in 1 grains was mapped on $4A^m$, three major QTLs for the roundness of 1 in 1 grains on $2A^m$, $5A^m$, and $7A^m$, one major QTL for the roundness of 2 in 1 grains on $6A^m$, two minor QTLs for the roundness of both 1 in 1 and 2 in 1 grains on $1A^m$ and $3A^m$, and four one minor QTLs for the length of either 1 in 1 or 2 in 1 grains on $2A^m$, $5A^m$, $6A^m$, and $7A^m$. The QTLs on all chromosomes except $5A^m$ overlap. The QTL on $1A^m$ mapped in a position similar to that with a QTL detected by Yu et al. (2019).

Grain thickness was calculated as the ratio of grain weight to grain area. Two major QTLs for the thickness of both 1 in 1 and 2 in 1 grains was mapped on $2A^m$ and $5A^m$, one major QTL for the thickness of 1 in 1 grains on $4A^m$, two minor QTLs for the thickness of both 1 in 1 and 2 in 1 grains on $3A^m$ and $7A^m$, and two minor QTLs for the thickness of 2 in 1 grains on $1A^m$ and $6A^m$. The QTLs on $2A^m$, $3A^m$, $5A^m$, and $7A^m$ overlap.

Generally, most of the QTLs for grain traits on chromosomes $1A^m$, $2A^m$, $4A^m$, $5A^m$, and $7A^m$ co-located at the same chromosomal regions. That is in agreement with Yu et al. (2019) who have also mapped QTLs for grain traits that clustered at certain chromosomal regions.

Another two grain shapes were analyzed ($\frac{\text{grain area}}{\text{grain width}}$ and $\frac{\text{grain area}}{\text{grain roundness}}$); however, both of them were found to be redundant as they strongly correlated with the basic grain parameters (grain area, length, and width) and were discarded.

Grain protein content is one of the important grain traits determining nutritional and end-use value of a harvested crop. Wheat is rich in carbohydrates but rather poor in protein (Vogel et al., 1976). Still, wheat is one of the main sources of worldwide protein supply; therefore, an improvement in grain protein content has been a major aim in wheat breeding programs focused on nutritional quality. However, grain protein content is negatively correlated with grain yield, which makes it more difficult to improve (Wang et al., 2012b). The QTLs for grain protein content were found on all chromosomes of both tetraploid and hexaploid wheat (see review by Kumar et al., 2018). Three major QTLs for grain protein content were mapped on $4A^m$, $5A^m$, and $7A^m$, and three minor QTLs on $1A^m$, $2A^m$, and $6A^m$.

The QTLs were mapped in chromosomal regions similar to those in which QTLs were detected in tetraploid and hexaploid wheat on 1A, 1B, 1D (Groos et al., 2003; Kulwal et al., 2005; Mann et al., 2009; Wang et al., 2012b; Deng et al., 2015; Tiwari et al., 2016b; Goel et al., 2019), 2A, 2B, 2D (Groos et al., 2003; Wang et al., 2012b; Deng et al., 2015; Maphosa et al., 2015; Giancaspro et al., 2019; Nigro et al., 2019), 4A, 4B (Zanetti et al., 2001; Groos et al., 2003; Blanco et al., 2012; Wang et al., 2012b; Fatiukha et al., 2019; Nigro et al., 2019), 5A, 5B, 5D (Blanco et al., 2002; Groos et al., 2003; Mann et al., 2009; Wang et al., 2012b; Deng et al., 2015; Fatiukha et al., 2019), 6A, 6B (Joppa et al., 1997; Groos et al., 2003; Breseghello et al., 2005; Peleg et al., 2009; Blanco et al., 2012; Fatiukha et al., 2019; Nigro et al., 2019), 7A, and 7B (Blanco et al., 2002; Groos et al., 2003; Blanco et al., 2012; Wang et al., 2012b; Nigro et al., 2019).

Taenzler et al. (2002) mapped QTLs for grain protein content on $1A^m$ and $5A^m$. The minor QTL mapped on $1A^m$ in the present study is in a similar position.

7 Conclusions

Triticum monococcum L. is closely related to *T. urartu*, the donor of the A-genome of common wheat, and is an attractive model for wheat genomic studies.

A linkage map of *T. monococcum* was constructed using 81 F_{12} RILs derived from a cross of cultivated *T. monococcum* ssp. *monococcum* 'DV92' and wild *T. monococcum* ssp. *boeoticum* 'G3116' (Dubcovsky et al., 1996; Vanžurová, 2013). The linkage map contains 676 molecular markers (338 skeleton and 338 attached markers) assigned to seven linkage groups corresponding to seven *T. monococcum* chromosomes and covers 1033 cM with one marker per 1.53 cM on average.

A total of 129 QTLs for 17 quantitative traits (plant height, the number of tillers, tillering pattern, leaf pubescence, ear emergence time, spike length, the number of spikelets per spike, spike compactness, rachis brittleness, the number of grains per spikelet, grain weight, grain area, grain width, grain length, grain roundness, grain thickness, and grain protein content) were detected using multiple environment multiple interval mapping. The highest number of QTLs was mapped on chromosome $5A^m$ (22), while the lowest on $6A^m$ (10). The QTLs for different traits often co-localized, especially on $2A^m$, $5A^m$, and $7A^m$. A sum of the average percentage of variance explained by QTLs for a trait ranged from 31% (the number of grains per spikelet) to 72% (leaf pubescence).

Leaf pubescence is a useful trait for breeders as it provides a protection against both biotic and abiotic stress factors. Two highly significant QTLs for leaf pubescence were mapped on $3A^m$ and $5A^m$ and the chromosomal regions with the QTLs on both chromosomes were saturated with markers. The sequences on which the markers associated with the QTLs are based on were BLASTN searched against the physical map of *T. aestivum* (IWGSC RefSeq v1.0; IWGSC, 2018). The markers for the QTL on $3A^m$ aligned within an interval between 709 and 729 Mbp on 3B and the markers for the QTL on $5A^m$ within an interval between 688.7 and 689.9 Mbp on 5A. Both QTLs were verified on verification mapping populations. This provides an ideal basis for the identification of underlying genes.

8 References

Acquaah, G. (2012). *Principles of plant genetics and breeding*. Wiley. ISBN: 978-0-470-66476-6

Adams, M., Kelley, J., Gocayne, J., Dubnick, M., Polymeropoulos, M., Xiao, H., Merril, C., Wu, A., Olde, B., & Moreno, R. (1991). Complementary DNA sequencing: Expressed sequence tags and human genome project. *Science*, *252*(5013). doi.org/10.1126/science.2047873

Alexandratos, N., & Bruinsma, J. (2012). *World agriculture towards 2030/2050: The 2012 revision. ESA Working paper No. 12-03*. Food and Agriculture Organization of the United Nations.

Altschul, S. (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research*, *25*(17). doi.org/10.1093/nar/25.17.3389

Alvarez, M. A., Tranquilli, G., Lewis, S., Kippes, N., & Dubcovsky, J. (2016). Genetic and physical mapping of the earliness *per se* locus *Eps-A^{m}1* in *Triticum monococcum* identifies *EARLY FLOWERING 3* (*ELF3*) as a candidate gene. *Functional & Integrative Genomics*, *16*(4). doi.org/10.1007/s10142-016-0490-3

Álvarez, M. F., Mosquera, T., & Blair, M. W. (2014). The use of association genetics approaches in plant breeding. In J. Janick (Ed.), *Plant Breeding Reviews, volume 38*. Wiley. doi.org/10.1002/9781118916865.ch02

Arzani, A., & Ashraf, M. (2017). Cultivated ancient wheats (*Triticum* spp.): A potential source of health-beneficial food products. *Comprehensive Reviews in Food Science and Food Safety*, *16*(3). doi.org/10.1111/1541-4337.12262

Assanga, S. O., Fuentealba, M., Zhang, G., Tan, C., Dhakal, S., Rudd, J. C., Ibrahim, A. M. H., Xue, Q., Haley, S., Chen, J., Chao, S., Baker, J., Jessup, K., & Liu, S. (2017). Mapping of quantitative trait loci for grain yield and its components in a US popular winter wheat TAM 111 using 90K SNPs. *PLOS ONE*, *12*(12). doi.org/10.1371/journal.pone.0189669

Bareš, I., Sehnalová, J., Vlasák, M., Vlach, M., Kryštof, Z., Amler, P., Malý, J., & Beránek, V. (1985). *Klasifikátor genus* Triticum *L.* Výzkumný ústav rostlinné výroby Praha-Ruzyně; Výzkumný a šlechtitelský ústav obilnářský Kroměříž.

Barkworth, M. E., Capels, K. M., Long, S., Anderton, L. K., & Piep, M. B. (Eds.). (2007). Magnoliophyta: Commelinidae (*in part*): Poaceae, *part 1* (Flora of North America: North of Mexico, Vol. 24). Oxford University Press. ISBN: 9780195167481

Barrett, B., Bayram, M., & Kidwell, K. (2002). Identifying AFLP and microsatellite markers for vernalization response gene *Vrn-B1* in hexaploid wheat using reciprocal mapping populations. *Plant Breeding*, *121*(5). doi.org/10.1046/j.1439-0523.2002.732319.x

Baulcombe, D. C. (1999). Fast forward genetics based on virus-induced gene silencing. *Current Opinion in Plant Biology*, *2*(2). doi.org/10.1016/s1369-5266(99)80022-3

Beales, J., Turner, A., Griffiths, S., Snape, J. W., & Laurie, D. A. (2007). A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *115*(5). doi.org/10.1007/s00122-007-0603-4

Bennett, D., Izanloo, A., Edwards, J., Kuchel, H., Chalmers, K., Tester, M., Reynolds, M., Schnurbusch, T., & Langridge, P. (2011). Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. *Theoretical and Applied Genetics*, *124*(4). doi.org/10.1007/s00122-011-1740-3

Bickford, C. P. (2016). Ecophysiology of leaf trichomes. *Functional Plant Biology*, *43*(9). doi.org/10.1071/fp16095

Blanco, A., Pasqualone, A., Troccoli, A., Di Fonzo, N., & Simeone, R. (2002). Detection of grain protein content QTLs across environments in tetraploid wheats. *Plant Molecular Biology*, *48*(5/6). doi.org/10.1023/a:1014864230933

Blanco, A., Mangini, G., Giancaspro, A., Giove, S., Colasuonno, P., Simeone, R., Signorile, A., De Vita, P., Mastrangelo, A. M., Cattivelli, L., & Gadaleta, A. (2012). Relationships between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. *Molecular Breeding*, *30*(1). doi.org/10.1007/s11032-011-9600-z

Boden, S. A., Cavanagh, C., Cullis, B. R., Ramm, K., Greenwood, J., Jean Finnegan, E., Trevaskis, B., & Swain, S. M. (2015). *Ppd-1* is a key regulator of inflorescence architecture and paired spikelet development in wheat. *Nature Plants*, *1*(2). doi.org/10.1038/nplants.2014.16

Borlaug, N. E. (1971). The Green Revolution: for bread and peace. *Bulletin of the Atomic Scientists*, *27*(6). doi.org/10.1080/00963402.1971.11455372

Börner, A., Schumann, E., Fürste, A., Cöster, H., Leithold, B., Röder, M., & Weber, W. (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 105(6), 921–936. doi.org/10.1007/s00122-002-0994-1

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*(3). ncbi.nlm.nih.gov/pmc/articles/PMC1686077/

Brandolini, A., Volante, A., & Heun, M. (2016). Geographic differentiation of domesticated einkorn wheat and possible Neolithic migration routes. *Heredity*, *117*(3). doi.org/10.1038/hdy.2016.32

Breseghello, F., Finney, P. L., Gaines, C., Andrews, L., Tanaka, J., Penner, G., & Sorrells, M. E. (2005). Genetic loci related to kernel quality differences between a soft and a hard wheat cultivar. *Crop Science*, *45*(5). doi.org/10.2135/cropsci2004.0310

Brinton, J., Simmonds, J., Minter, F., Leverington-Waite, M., Snape, J., & Uauy, C. (2017). Increased pericarp cell length underlies a major quantitative trait locus for grain weight in hexaploid wheat. *New Phytologist*, *215*(3). doi.org/10.1111/nph.14624

Broman, K. W., Wu, H., Sen, S., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics*, *19*(7). doi.org/10.1093/bioinformatics/btg112

Buckler, E. S., & Thornsberry, J. M. (2002). Plant molecular diversity and applications to genomics. *Current Opinion in Plant Biology*, *5*(2). doi.org/10.1016/s1369-5266(02)00238-8

Bullrich, L., Appendino, M., Tranquilli, G., Lewis, S., & Dubcovsky, J. (2002). Mapping of a thermo-sensitive earliness *per se* gene on *Triticum monococcum* chromosome $1A^m$. *Theoretical and Applied Genetics*, *105*(4). doi.org/10.1007/s00122-002-0982-5

Cabral, A. L., Jordan, M. C., Larson, G., Somers, D. J., Humphreys, D. G., & McCartney, C. A. (2018). Relationship between QTL for grain shape, grain weight, test weight, milling yield, and plant height in the spring wheat cross RL4452/'AC Domain.' *PLOS ONE*, *13*(1). doi.org/10.1371/journal.pone.0190681

Cardoso, M. Z. (2008). Herbivore handling of a plant's trichome: The case of *Heliconius charithonia* (L.) (*Lepidoptera*: *Nymphalidae*) and *Passiflora lobata* (Killip) Hutch. (*Passifloraceae*). *Neotropical Entomology*, *37*(3). doi.org/10.1590/s1519-566x2008000300002

Carter, T. C., & Falconer, D. S. (1951). Stocks for detecting linkage in the mouse, and the theory of their design. *Journal of Genetics*, *50*(2). doi.org/10.1007/bf02996226

Cavanagh, C., Morell, M., Mackay, I., & Powell, W. (2008). From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. *Current Opinion in Plant Biology*, *11*(2). doi.org/10.1016/j.pbi.2008.01.002

Chalupska, D., Lee, H. Y., Faris, J. D., Evrard, A., Chalhoub, B., Haselkorn, R., & Gornicki, P. (2008). Acc homoeoloci and the evolution of wheat genomes. *Proceedings of the National Academy of Sciences*, *105*(28). doi.org/10.1073/pnas.0803981105

Chen, Q.-F., Yen, C., & Yang, J.-L. (1999). Chromosome location of the gene for the hulled character in the Tibetan weedrace of common wheat. *Genetic Resources and Crop Evolution*, *46*(6). doi.org/10.1023/a:1008738423817

Chen, Z., Zheng, G., Ghosh, K., & Li, Z. (2005). Linkage disequilibrium mapping of quantitative-trait loci by selective genotyping. *The American Journal of Human Genetics*, *77*(4). doi.org/10.1086/491658

Chen, L., Huang, L., Min, D., Phillips, A., Wang, S., Madgwick, P. J., Parry, M. A. J., & Hu, Y.-G. (2012). Development and characterization of a new TILLING population of common bread wheat (*Triticum aestivum* L.). *PloS One*, *7*(7). doi.org/10.1371/journal.pone.0041570

Chen, Z. (2014). *Statistical methods for QTL mapping*. CRC Press/Taylor & Francis Group. ISBN: 9781439868317

Chen, Z., Cheng, X., Chai, L., Wang, Z., Du, D., Wang, Z., Bian, R., Zhao, A., Xin, M., Guo, W., Hu, Z., Peng, H., Yao, Y., Sun, Q., & Ni, Z. (2020). Pleiotropic QTL influencing spikelet number and heading date in common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*. doi.org/10.1007/s00122-020-03556-6

Choi, Y.-E., Harada, E., Wada, M., Tsuboi, H., Morita, Y., Kusano, T., & Sano, H. (2001). Detoxification of cadmium in tobacco plants: formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta*, *213*(1). doi.org/10.1007/s004250000487

Chu, C.-G., Xu, S. S., Friesen, T. L., & Faris, J. D. (2008). Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. *Molecular Breeding*, *22*(2). doi.org/10.1007/s11032-008-9171-9

Churchill, G. A., & Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, *138*(3). ncbi.nlm.nih.gov/pmc/articles/PMC1206241/

Cockram, J., White, J., Zuluaga, D. L., Smith, D., Comadran, J., Macaulay, M., Luo, Z., Kearsey, M. J., Werner, P., Harrap, D., Tapsell, C., Liu, H., Hedley, P. E., Stein, N., Schulte, D., Steuernagel, B., Marshall, D. F., Thomas, W. T. B., Ramsay, L., … Wang, M. (2010). Genome-wide association mapping to candidate polymorphism resolution in the unsequenced barley genome. *Proceedings of the National Academy of Sciences*, *107*(50). doi.org/10.1073/pnas.1010179107

Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., & Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, *339*(6121). doi.org/10.1126/science.1231143

Cui, F., Ding, A., Li, J., Zhao, C., Wang, L., Wang, X., Qi, X., Li, X., Li, G., Gao, J., & Wang, H. (2011). QTL detection of seven spike-related traits and their genetic correlations in wheat using two related RIL populations. *Euphytica*, *186*(1). doi.org/10.1007/s10681-011-0550-7

Cuthbert, J. L., Somers, D. J., Brûlé-Babel, A. L., Brown, P. D., & Crow, G. H. (2008). Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *117*(4). doi.org/10.1007/s00122-008-0804-5

Das, S., Upadhyaya, H. D., Bajaj, D., Kujur, A., Badoni, S., Laxmi, Kumar, V., Tripathi, S., Gowda, C. L. L., Sharma, S., Singh, S., Tyagi, A. K., & Parida, S. K. (2015). Deploying QTL-seq for rapid delineation of a potential candidate gene underlying major trait-associated QTL in chickpea. *DNA Research*, *22*(3). doi.org/10.1093/dnares/dsv004

Deng, Z., Hu, S., Chen, F., Li, W., Chen, J., Sun, C., Zhang, Y., Wang, S., Song, X., & Tian, J. (2015). Genetic dissection of interaction between wheat protein and starch using three mapping populations. *Molecular Breeding*, *35*(1). doi.org/10.1007/s11032-015-0216-6

Deng, Z., Cui, Y., Han, Q., Fang, W., Li, J., & Tian, J. (2017). Discovery of consistent QTLs of wheat spike-related traits under nitrogen treatment at different development stages. *Frontiers in Plant Science*, *8*(2120). doi.org/10.3389/fpls.2017.02120

Deschamps, C., Gang, D., Dudareva, N., & Simon, J. E. (2006). Developmental regulation of phenylpropanoid biosynthesis in leaves and glandular trichomes of basil (*Ocimum basilicum* L.). *International Journal of Plant Sciences*, *167*(3). doi.org/10.1086/500987

Devos, K. M., Atkinson, M. D., Chinoy, C. N., Francis, H. A., Harcourt, R. L., Koebner, R. M. D., Liu, C. J., Masojć, P., Xie, D. X., & Gale, M. D. (1993). Chromosomal rearrangements in the rye genome relative to that of wheat. *Theoretical and Applied Genetics*, *85–85*(6–7). doi.org/10.1007/bf00225004

Dimock, M. B., & Kennedy, G. G. (1983). The role of glandular trichomes in the resistance of *Lycopersicon hirsutum* f. *Glabratum* to *Heliothis zea*. *Entomologia Experimentalis et Applicata*, *33*(3). doi.org/10.1111/j.1570-7458.1983.tb03267.x

Dobrovolskaya, O., Pshenichnikova, T. A., Arbuzova, V. S., Lohwasser, U., Röder, M. S., & Börner, A. (2007). Molecular mapping of genes determining hairy leaf character in common wheat with respect to other species of the *Triticeae*. *Euphytica*, *155*(3). doi.org/10.1007/s10681-006-9329-7

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics*, *3*(1). doi.org/10.1038/nrg703

Doroshkov, A. V., Afonnikov, D. A., & Pshenichnikova, T. A. (2014). Genetic analysis of leaf pubescence in isogenic lines of bread wheat Novosibirskaya 67. *Russian Journal of Genetics*, *50*(2). doi.org/10.1134/s1022795413120028

Doroshkov, A. V., Afonnikov, D. A., Dobrovolskaya, O. B., & Pshenichnikova, T. A. (2015). Interactions between leaf pubescence genes in bread wheat as assessed by high throughput phenotyping. *Euphytica*, *207*(3). doi.org/10.1007/s10681-015-1520-2

Dubcovsky, J., Luo, M.-C., Zhong, G. Y., Bransteitter, R., Desai, A., Kilian, A., Kleinhofs, A., & Dvořák, J. (1996). Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. *Genetics*, *143*(2). ncbi.nlm.nih.gov/pmc/articles/PMC1207354/

Dubcovsky, J., Lijavetzky, D., Appendino, L., & Tranquilli, G. (1998). Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. *Theoretical and Applied Genetics*, *97*(5–6). doi.org/10.1007/s001220050978

Dvořák, J., McGuire, P. E., & Cassidy, B. (1988). Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences. *Genome*, *30*(5). doi.org/10.1139/g88-115

Dvořák, J., & Zhang, H.-B. (1990). Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. *Proceedings of the National Academy of Sciences*, *87*(24). doi.org/10.1073/pnas.87.24.9640

Dvořák, J., Terlizzi, P., Zhang, H.-B., & Resta, P. (1993). The evolution of polyploid wheats: identification of the A genome donor species. *Genome*, *36*(1). doi.org/10.1139/g93-004

Dvořák, J., & Akhunov, E. D. (2005). Tempos of gene locus deletions and duplications and their relationship to recombination rate during diploid and polyploid evolution in the Aegilops-Triticum alliance. *Genetics*, *171*(1). doi.org/10.1534/genetics.105.041632

Dvořák, J., Deal, K. R., Luo, M.-C., You, F. M., von Borstel, K., & Dehghani, H. (2012). The origin of spelt and free-threshing hexaploid wheat. *Journal of Heredity*, *103*(3). doi.org/10.1093/jhered/esr152

Echeverry-Solarte, M., Kumar, A., Kianian, S., Mantovani, E. E., McClean, P. E., Deckard, E. L., Elias, E., Simsek, S., Alamri, M. S., Hegstad, J., Schatz, B., & Mergoum, M. (2015). Genome-wide mapping of spike-related and agronomic traits in a common wheat population derived from a supernumerary spikelet parent and an elite parent. *The Plant Genome*, *8*(2). doi.org/10.3835/plantgenome2014.12.0089

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5). doi.org/10.1093/nar/gkh340

Fahn, A. (2000). Structure and function of secretory cells. *Advances in Botanical Research*, *31*. doi.org/10.1016/s0065-2296(00)31006-0

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Pearson. ISBN: 9780582243026

Fan, X., Cui, F., Ji, J., Zhang, W., Zhao, X., Liu, J., Meng, D., Tong, Y., Wang, T., & Li, J. (2019). Dissection of pleiotropic QTL regions controlling wheat spike characteristics under different nitrogen treatments using traditional and conditional QTL mapping. *Frontiers in Plant Science*, *10*(187). doi.org/10.3389/fpls.2019.00187

Faris, J. D., & Gill, B. S. (2002). Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. *Genome*, *45*(4). doi.org/10.1139/g02-036

Faris, J. D., Zhang, Q., Chao, S., Zhang, Z., & Xu, S. S. (2014). Analysis of agronomic and domestication traits in a durum × cultivated emmer wheat population using a high-density single nucleotide polymorphism-based linkage map. *Theoretical and Applied Genetics*, *127*(11). doi.org/10.1007/s00122-014-2380-1

Fatiukha, A., Filler, N., Lupo, I., Lidzbarsky, G., Klymiuk, V., Korol, A. B., Pozniak, C., Fahima, T., & Krugman, T. (2019). Grain protein content and thousand kernel weight QTLs identified in a durum × wild emmer wheat mapping population tested in five environments. *Theoretical and Applied Genetics*, *133*(1). doi.org/10.1007/s00122-019-03444-8

Felsenstein, J. (1979). A mathematically tractable family of genetic mapping functions with different amounts of interference. *Genetics*, *91*(4). genetics.org/content/91/4/769

Feuillet, C., Langridge, P., & Waugh, R. (2008). Cereal breeding takes a walk on the wild side. *Trends in Genetics*, *24*(1), 24–32. doi.org/10.1016/j.tig.2007.11.001

Fisher, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Philosophical Transactions of the Royal Society of Edinburgh*, *52*(2). doi.org/10.1017/s0080456800012163

Fisher, R. A. (1935). *The Design of Experiments*. Oliver & Boyd.

Flint-Garcia, S. A., Thornsberry, J. M., & Buckler, E. S. (2003). Structure of linkage disequilibrium in plants. *Annual Review of Plant Biology*, *54*(1). doi.org/10.1146/annurev.arplant.54.031902.134907

Franckowiak, J. D. (1997). Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. *Barley Genetics Newsletter*, *26*.

Fulker, D. W., Cherny, S. S., Sham, P. C., & Hewitt, J. K. (1999). Combined linkage and association sib-pair analysis for quantitative traits. *The American Journal of Human Genetics*, *64*(1). doi.org/10.1086/302193

Fuller, D. Q. (2007). Contrasting patterns in crop domestication and domestication rates: Recent archaeobotanical insights from the Old World. *Annals of Botany*, *100*(5). doi.org/10.1093/aob/mcm048

Gallová (Hlusková), L. (2011). *Mapovanie agronomicky dôležitých génov u pšenice*. Přírodovědecká fakulta Univerzity Palackého. theses.cz/id/ybxc1y

Gallun, R. L., Ruppel, R., & Everson, E. H. (1966). Resistance of small grains to the cereal leaf beetle. *Journal of Economic Entomology*, *59*(4). doi.org/10.1093/jee/59.4.827

Gao, F., Wen, W., Liu, J., Rasheed, A., Yin, G., Xia, X., Wu, X., & He, Z. (2015). Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the Chinese wheat cross Zhou 8425B/Chinese Spring. *Frontiers in Plant Science*, *6*(1099). doi.org/10.3389/fpls.2015.01099

Gasperini, D., Greenland, A., Hedden, P., Dreos, R., Harwood, W., & Griffiths, S. (2012). Genetic and physiological analysis of *Rht8* in bread wheat: An alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. *Journal of Experimental Botany*, *63*(12). doi.org/10.1093/jxb/ers138

Gegas, V. C., Nazari, A., Griffiths, S., Simmonds, J., Fish, L., Orford, S., Sayers, L., Doonan, J. H., & Snape, J. W. (2010). A genetic framework for grain size and shape variation in wheat. *The Plant Cell*, *22*(4). doi.org/10.1105/tpc.110.074153

Genaev, M. A., Doroshkov, A. V., Pshenichnikova, T. A., Kolchanov, N. A., & Afonnikov, D. A. (2012). Extraction of quantitative characteristics describing wheat leaf pubescence with a novel image-processing technique. *Planta*, *236*(6). doi.org/10.1007/s00425-012-1751-6

Gershenzon, J., McCaskill, D., Rajaonarivony, J. I. M., Mihaliak, C., Karp, F., & Croteau, R. (1992). Isolation of secretory cells from plant glandular trichomes and their use in biosynthetic studies of monoterpenes and other gland products. *Analytical Biochemistry*, *200*(1). doi.org/10.1016/0003-2697(92)90288-i

Gershenzon, J., & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology*, *3*(7). doi.org/10.1038/nchembio.2007.5

Ghorashy, S. R., Pendleton, J. W., Bernard, R. L., & Bauer, M. E. (1971). Effect of leaf pubescence on transpiration, photosynthetic rate and seed yield of three near-isogenic lines of soybeans. *Crop Science*, *11*(3). doi.org/10.2135/cropsci1971.0011183x001100030035x

Giancaspro, A., Giove, S. L., Zacheo, S. A., Blanco, A., & Gadaleta, A. (2019). Genetic variation for protein content and yield-related traits in a durum population derived from an inter-specific cross between hexaploid and tetraploid wheat cultivars. *Frontiers in Plant Science*, *10*(1509). doi.org/10.3389/fpls.2019.01509

Glas, J., Schimmel, B., Alba, J., Escobar-Bravo, R., Schuurink, R., & Kant, M. (2012). Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. *International Journal of Molecular Sciences*, *13*(12). doi.org/10.3390/ijms131217077

Goel, S., Singh, K., Singh, B., Grewal, S., Dwivedi, N., Alqarawi, A. A., Abd_Allah, E. F., Ahmad, P., & Singh, N. K. (2019). Analysis of genetic control and QTL mapping of essential wheat grain quality traits in a recombinant inbred population. *PLOS ONE*, *14*(3). doi.org/10.1371/journal.pone.0200669

Gosset, W. S. (1908). The probable error of a mean. *Biometrika*, *6*(1). doi.org/10.2307/2331554

Griffiths, S., Simmonds, J., Leverington, M., Wang, Y., Fish, L., Sayers, L., Alibert, L., Orford, S., Wingen, L., Herry, L., Faure, S., Laurie, D., Bilham, L., & Snape, J. (2009). Meta-QTL analysis of the genetic control of ear emergence in elite European winter wheat germplasm. *Theoretical and Applied Genetics*, *119*(3). doi.org/10.1007/s00122-009-1046-x

Groos, C., Robert, N., Bervas, E., & Charmet, G. (2003). Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics*, *106*(6). doi.org/10.1007/s00122-002-1111-1

Guo, Z., Chen, D., Alqudah, A. M., Röder, M. S., Ganal, M. W., & Schnurbusch, T. (2016). Genome-wide association analyses of 54 traits identified multiple loci for the determination of floret fertility in wheat. *New Phytologist*, *214*(1). doi.org/10.1111/nph.14342

Haldane, J. B. S. (1919). The combination of linkage values and the calculation of distance between the loci of linked factors. *Journal of Genetics*, *8*.

Haldorsen, S., Akan, H., Çelik, B., & Heun, M. (2011). The climate of the Younger Dryas as a boundary for einkorn domestication. *Vegetation History and Archaeobotany*, *20*. doi.org/10.1007/s00334-011-0291-5

Haley, C. S., & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*(4). doi.org/10.1038/hdy.1992.131

Hanin, M., & Paszkowski, J. (2003). Plant genome modification by homologous recombination. *Current Opinion in Plant Biology*, *6*(2). doi.org/10.1016/s1369-5266(03)00016-5

Harlan, J. R., & Zohary, D. (1966). Distribution of wild wheats and barley. *Science*, *153*(3740). doi.org/10.1126/science.153.3740.1074

Harvey, T. L., & Martin, T. J. (1980). Effects of wheat pubescence on infestations of wheat curl mite and incidence of wheat streak mosaic. *Journal of Economic Entomology*, *73*(2). doi.org/10.1093/jee/73.2.225

Hayes, P. M., Liu, B. H., Knapp, S. J., Chen, F., Jones, B., Blake, T., Franckowiak, J., Rasmusson, D., Sorrells, M., Ullrich, S. E., Wesenberg, D., & Kleinhofs, A. (1993). Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. *Theoretical and Applied Genetics*, *87*(3). doi.org/10.1007/bf01184929

Hearne, C., Ghosh, S., & Todd, J. (1992). Microsatellites for linkage analysis of genetic traits. *Trends in Genetics*, *8*(8). doi.org/10.1016/0168-9525(92)90256-4

Heun, M. (1997). Site of einkorn wheat domestication identified by DNA fingerprinting. *Science*, *278*(5341). doi.org/10.1126/science.278.5341.1312

Hong, Y., Chen, L., Du, L., Su, Z., Wang, J., Ye, X., Qi, L., & Zhang, Z. (2014). Transcript suppression of *TaGW2* increased grain width and weight in bread wheat. *Functional & Integrative Genomics*, *14*(2). doi.org/10.1007/s10142-014-0380-5

Hori, K., Takehara, S., Nankaku, N., Sato, K., Sasakuma, T., & Takeda, K. (2007). Barley EST markers enhance map saturation and QTL mapping in diploid wheat. *Breeding Science*, *57*(1). doi.org/10.1270/jsbbs.57.39

Hosseini Tafreshi, S. A., Shariati, M., Mofid, M. R., Khayam Nekui, M., & Esmaeili, A. (2011). Heterologous virus-induced gene silencing as a promising approach in plant functional genomics. *Molecular Biology Reports*, *39*(3). doi.org/10.1007/s11033-011-0965-1

Huang, X., Börner, A., Röder, M., & Ganal, M. (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*, *105*(5). doi.org/10.1007/s00122-002-0959-4

Huang, X. Q., Cöster, H., Ganal, M. W., & Röder, M. S. (2003). Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *106*(8). doi.org/10.1007/s00122-002-1179-7

Huang, X. Q., Kempf, H., Ganal, M. W., & Röder, M. S. (2004). Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *109*(5). doi.org/10.1007/s00122-004-1708-7

IWGSC. (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science*, *361*(6403). doi.org/10.1126/science.aar7191

Jaccoud, D. (2001). Diversity Arrays: A solid-state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*(4). doi.org/10.1093/nar/29.4.e25

Jansen, R. C., & Stam, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, *136*(4). ncbi.nlm.nih.gov/pmc/articles/PMC1205923/

Jantasuriyarat, C., Vales, M. I., Watson, C. J. W., & Riera-Lizarazu, O. (2003). Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *108*(2). doi.org/10.1007/s00122-003-1432-8

Jiang, Y.-F., Lan, X.-J., Luo, W., Kong, X.-C., Qi, P.-F., Wang, J.-R., Wei, Y.-M., Jiang, Q.-T., Liu, Y.-X., Peng, Y.-Y., Chen, G.-Y., Dai, S.-F., & Zheng, Y.-L. (2014). Genome-wide quantitative trait locus mapping identifies multiple major loci for brittle rachis and threshability in Tibetan semi-wild wheat (*Triticum aestivum* ssp. *Tibetanum* Shao). *PLoS ONE*, *9*(12). doi.org/10.1371/journal.pone.0114066

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, *337*(6096). doi.org/10.1126/science.1225829

Jing, H.-C., Kornyukhin, D., Kanyuka, K., Orford, S., Zlatska, A., Mitrofanova, O. P., Koebner, R., & Hammond-Kosack, K. (2007). Identification of variation in adaptively important traits and genome-wide analysis of trait–marker associations in *Triticum monococcum*. *Journal of Experimental Botany*, *58*(13). doi.org/10.1093/jxb/erm225

Jing, H.-C., Bayon, C., Kanyuka, K., Berry, S., Wenzl, P., Huttner, E., Kilian, A., & E Hammond-Kosack, K. (2009). DArT markers: Diversity analyses, genomes comparison, mapping and integration with SSR markers in *Triticum monococcum*. *BMC Genomics*, *10*(1). doi.org/10.1186/1471-2164-10-458

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics*, *24*(23). doi.org/10.1093/bioinformatics/btn523

Johnson, E. B., Nalam, V. J., Zemetra, R. S., & Riera-Lizarazu, O. (2007). Mapping the compactum locus in wheat (*Triticum aestivum* L.) and its relationship to other spike morphology genes of the *Triticeae*. *Euphytica*, *163*(2). doi.org/10.1007/s10681-007-9628-7

Joppa, L. R., Du, C., Hart, G. E., & Hareland, G. A. (1997). Mapping gene(s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines. *Crop Science*, *37*(5). doi.org/10.2135/cropsci1997.0011183x003700050030x

Kadkol, G., & Sissons, M. (2016). Durum wheat: Overview. In C. Wrigley, H. Corke, J. Faubion, & K. Seetharaman (Eds.), *Encyclopedia of food grains*. UK Academic Press. doi.org/10.1016/B978-0-08-100596-5.00024-X

Kao, C.-H., Zeng, Z.-B., & Teasdale, R. D. (1999). Multiple interval mapping for quantitative trait loci. *Genetics*, *152*(3). ncbi.nlm.nih.gov/pmc/articles/PMC1460657/

Karabourniotis, G., Kotsabassidis, D., & Manetas, Y. (1995). Trichome density and its protective potential against ultraviolet-B radiation damage during leaf development. *Canadian Journal of Botany*, *73*(3). doi.org/10.1139/b95-039

Katkout, M., Kishii, M., Kawaura, K., Mishina, K., Sakuma, S., Umeda, K., Takumi, S., Nitta, M., Nasuda, S., & Ogihara, Y. (2014). QTL analysis of genetic loci affecting domestication-related spike characters in common wheat. *Genes & Genetic Systems*, *89*(3). doi.org/10.1266/ggs.89.121

Kato, K., Miura, H., & Sawada, S. (1999). QTL mapping of genes controlling ear emergence time and plant height on chromosome 5A of wheat. *Theoretical and Applied Genetics*, *98*(3–4). doi.org/10.1007/s001220051094

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12). doi.org/10.1093/bioinformatics/bts199

Kearsey, M. J., & Hyne, V. (1994). QTL analysis: A simple 'marker-regression' approach. *Theoretical and Applied Genetics*, *89*(6). doi.org/10.1007/bf00223708

Kebrom, T. H., Chandler, P. M., Swain, S. M., King, R. W., Richards, R. A., & Spielmeyer, W. (2012). Inhibition of tiller bud outgrowth in the *tin* mutant of wheat is associated with precocious internode development. *Plant Physiology*, *160*(1). doi.org/10.1104/pp.112.197954

Khlestkina, E. K., Pestsova, E. G., Salina, E., Röder, M. S., Arbuzova, V. S., Koval, S. F., & Börner, A. (2002). Genetic mapping and tagging of wheat genes using RAPD, STS and SSR markers. *Cellular & Molecular Biology Letters*, *7*(2B).

Kihara, H. (1944). Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare*. *Agriculture and Horticulture*, *19*.

Kilian, B., Ozkan, H., Walther, A., Kohl, J., Dagan, T., Salamini, F., & Martin, W. (2007). Molecular diversity at 18 loci in 321 wild and 92 domesticate lines reveal no reduction of nucleotide diversity during *Triticum monococcum* (einkorn) domestication: Implications for the origin of agriculture. *Molecular Biology and Evolution*, *24*(12). doi.org/10.1093/molbev/msm192

Klocová (Balcárková), B. (2010). *Mapování genů kvantitativních znaků u diploidní pšenice* T. monococcum *L.* Přírodovědecká fakulta Univerzity Palackého. theses.cz/id/p1eq9j

Kojima, T., Nagaoka, T., Noda, K., & Ogihara, Y. (1998). Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. Theoretical and Applied Genetics, 96(1), 37–45. doi.org/10.1007/s001220050706

Konrad, W., Burkhardt, J., Ebner, M., & Roth-Nebelsick, A. (2014). Leaf pubescence as a possibility to increase water use efficiency by promoting condensation. *Ecohydrology*, *8*(3). doi.org/10.1002/eco.1518

Korol, A. B., Ronin, Y. I., Itskovich, A. M., Peng, J., & Nevo, E. (2001). Enhanced efficiency of quantitative trait loci mapping analysis based on multivariate complexes of quantitative traits. *Genetics*, *157*(4). ncbi.nlm.nih.gov/pmc/articles/PMC1461583/

Korzun, V., Börner, A., & Melz, G. (1996). RFLP mapping of the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5 of rye (*Secale cereale* L.). *Theoretical and Applied Genetics*, *92*(8). doi.org/10.1007/bf00224051

Korzun, V., Röder, M. S., Ganal, M. W., Worland, A. J., & Law, C. N. (1998). Genetic analysis of the dwarfing gene (*Rht8*) in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *96*(8). doi.org/10.1007/s001220050845

Korzun, V., Malyshev, S., Pickering, R. A., & Börner, A. (1999). RFLP mapping of a gene for hairy leaf sheath using a recombinant line from *Hordeum vulgare* L. × *Hordeum bulbosum* L. cross. *Genome*, *42*(5). doi.org/10.1139/g99-021

Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics*, *12*(1). doi.org/10.1111/j.1469-1809.1943.tb02321.x

Kruepl, C., Hoad, S., Davies, K., Bertholdsson, N.-O., & Paolini, R. (2006). Weed competitiveness. In D. Donner & A. Osman (Eds.), *Handbook on cereal variety testing for organic and low input agriculture*. COST860-SUSVAR.

Kuchel, H., Hollamby, G., Langridge, P., Williams, K., & Jefferies, S. P. (2006). Identification of genetic loci associated with ear-emergence in bread wheat. *Theoretical and Applied Genetics*, *113*(6). doi.org/10.1007/s00122-006-0370-7

Kulwal, P., Kumar, N., Kumar, A., Gupta, R. K., Balyan, H. S., & Gupta, P. K. (2005). Gene networks in hexaploid wheat: Interacting quantitative trait loci for grain protein content. *Functional & Integrative Genomics*, *5*(4). doi.org/10.1007/s10142-005-0136-3

Kumar, N., Kulwal, P. L., Balyan, H. S., & Gupta, P. K. (2006). QTL mapping for yield and yield contributing traits in two mapping populations of bread wheat. *Molecular Breeding*, *19*(2). doi.org/10.1007/s11032-006-9056-8

Kumar, A., Mantovani, E. E., Seetan, R., Soltani, A., Echeverry-Solarte, M., Jain, S., Simsek, S., Doehlert, D., Alamri, M. S., Elias, E. M., Kianian, S. F., & Mergoum, M. (2016). Dissection of genetic factors underlying wheat kernel shape and size in an elite × nonadapted cross using a high density SNP linkage map. *The Plant Genome*, *9*(1). doi.org/10.3835/plantgenome2015.09.0081

Kumar, A, Jain, S., Elias, E. M., Ibrahim, M., & Sharma, L. K. (2018). An overview of QTL identification and marker-assisted selection for grain protein content in wheat. In R. S. Sengar & A. Singh (Eds.), *Eco-friendly agro-biological techniques for enhancing crop productivity*. Springer. ISBN: 978-981-10-6934-5

Kumari, S., Mir, R. R., Tyagi, S., Balyan, H. S., & Gupta, P. K. (2019). Validation of QTL for grain weight using MAS-derived pairs of NILs in bread wheat (*Triticum aestivum* L.). *Journal of Plant Biochemistry and Biotechnology*, *28*(3). doi.org/10.1007/s13562-018-0485-3

Kuraparthy, V., Sood, S., Dhaliwal, H. S., Chhuneja, P., & Gill, B. S. (2006). Identification and mapping of a tiller inhibition gene (*tin3*) in wheat. *Theoretical and Applied Genetics*, *114*(2). doi.org/10.1007/s00122-006-0431-y

Kusaba, M. (2004). RNA interference in crop plants. *Current Opinion in Biotechnology*, *15*(2). doi.org/10.1016/j.copbio.2004.02.004

Kuspira, J., Maclagan, J., Bhambhani, R. N., Sadasivaiah, R. S., & Kim, N.-S. (1989). Genetic and cytogenetic analyses of the A genome of *Triticum monococcum* L. V. Inheritance and linkage relationships of genes determining the expression of 12 qualitative characters. *Genome*, *32*(5). doi.org/10.1139/g89-524

Kuzay, S., Xu, Y., Zhang, J., Katz, A., Pearce, S., Su, Z., Fraser, M., Anderson, J. A., Brown-Guedira, G., DeWitt, N., Peters Haugrud, A., Faris, J. D., Akhunov, E., Bai, G., & Dubcovsky, J. (2019). Identification of a candidate gene for a QTL for spikelet number per spike on wheat chromosome arm 7AL by high-resolution genetic mapping. *Theoretical and Applied Genetics*, *132*(9). doi.org/10.1007/s00122-019-03382-5

Lampar, A. (2018). *Mapování a charakterizace lokusů ovlivňujících důležité agronomické znaky pšenice*. Přírodovědecká fakulta Univerzity Palackého. theses.cz/id/g9zpfv

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., & Newburg, L. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, *1*(2). doi.org/10.1016/0888-7543(87)90010-3

Lander, E. S., & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*(1). ncbi.nlm.nih.gov/pmc/articles/PMC1203601/

Lander, E. S., & Schork, N. (1994). Genetic dissection of complex traits. *Science*, *265*(5181). doi.org/10.1126/science.8091226

Lander, E. S., & Kruglyak, L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nature Genetics*, *11*(3). doi.org/10.1038/ng1195-241

Larsson, S. J., Lipka, A. E., & Buckler, E. S. (2013). Lessons from *Dwarf8* on the strengths and weaknesses of structured association mapping. *PLoS Genetics*, *9*(2). doi.org/10.1371/journal.pgen.1003246

Laurie, D. A., & Bennett, M. D. (1988). The production of haploid wheat plants from wheat × maize crosses. *Theoretical and Applied Genetics*, *76*(3). doi.org/10.1007/bf00265339

Lebreton, C. M., & Visscher, P. M. (1998). Empirical nonparametric bootstrap strategies in quantitative trait loci mapping: Conditioning on the genetic model. *Genetics*, *148*(1). ncbi.nlm.nih.gov/pubmed/9475761

Levin, D. A. (1973). The role of trichomes in plant defense. *The Quarterly Review of Biology*, *48*(1). jstor.org/stable/2822621

Lewis, S., Faricelli, M. E., Appendino, M. L., Valárik, M., & Dubcovsky, J. (2008). The chromosome region including the earliness *per se* locus *Eps-A^{m}1* affects the duration of early developmental phases and spikelet number in diploid wheat. *Journal of Experimental Botany*, *59*(13). doi.org/10.1093/jxb/ern209

Li, W., & Gill, B. S. (2006). Multiple genetic pathways for seed shattering in the grasses. *Functional & Integrative Genomics*, *6*(4). doi.org/10.1007/s10142-005-0015-y

Li, W. L., Nelson, J. C., Chu, C. Y., Shi, L. H., Huang, S. H., & Liu, D. J. (2002). Chromosomal locations and genetic relationships of tiller and spike characters in wheat. *Euphytica*, *125*(3). doi.org/10.1023/a:1016069809977

Li, H., Ye, G., & Wang, J. (2006). A modified algorithm for the improvement of composite interval mapping. *Genetics*, *175*(1). doi.org/10.1534/genetics.106.066811

Li, C., Bai, G., Carver, B. F., Chao, S., & Wang, Z. (2015). Mapping quantitative trait loci for plant adaptation and morphology traits in wheat using single nucleotide polymorphisms. *Euphytica*, *208*(2). doi.org/10.1007/s10681-015-1594-x

Li, W., & Yang, B. (2017). Translational genomics of grain size regulation in wheat. *Theoretical and Applied Genetics*, *130*(9). doi.org/10.1007/s00122-017-2953-x

Lincoln, S. E., Daly, M. J., & Lander, E. S. (1993). *Mapping genes controlling quantitative traits using MAPMAKER/QTL version 1.1: A tutorial and reference manual* (2nd ed.). A Whitehead Institute for Biomedical Research Technical Report.

Liu, Y.-G., Shirano, Y., Fukaki, H., Yanai, Y., Tasaka, M., Tabata, S., & Shibata, D. (1999). Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. *Proceedings of the National Academy of Sciences*, *96*(11). doi.org/10.1073/pnas.96.11.6535

Liu, Y., Schiff, M., & Dinesh-Kumar, S. P. (2002). Virus-induced gene silencing in tomato. *The Plant Journal*, *31*(6). doi.org/10.1046/j.1365-313x.2002.01394.x

Liu, G., Jia, L., Lu, L., Qin, D., Zhang, J., Guan, P., Ni, Z., Yao, Y., Sun, Q., & Peng, H. (2014). Mapping QTLs of yield-related traits using RIL population derived from common wheat and Tibetan semi-wild wheat. *Theoretical and Applied Genetics*, *127*(11). doi.org/10.1007/s00122-014-2387-7

Liu, J., Luo, W., Qin, N., Ding, P., Zhang, H., Yang, C., Mu, Y., Tang, H., Liu, Y., Li, W., Jiang, Q., Chen, G., Wei, Y., Zheng, Y., Liu, C., Lan, X., & Ma, J. (2018a). A 55 K SNP array-based genetic map and its utilization in QTL mapping for productive tiller number in common wheat. *Theoretical and Applied Genetics*, *131*(11). doi.org/10.1007/s00122-018-3164-9

Liu, J., Xu, Z., Fan, X., Zhou, Q., Cao, J., Wang, F., Ji, G., Yang, L., Feng, B., & Wang, T. (2018b). A genome-wide association study of wheat spike related traits in China. *Frontiers in Plant Science*, *9*(1584). doi.org/10.3389/fpls.2018.01584

Liu, H., Ma, J., Tu, Y., Zhu, J., Ding, P., Liu, J., Li, T., Zou, Y., Habib, A., Mu, Y., Tang, H., Jiang, Q., Liu, Y., Chen, G., Zheng, Y., Wei, Y., & Lan, X. (2019). Several stably expressed QTL for spike density of common wheat (*Triticum aestivum*) in multiple environments. *Plant Breeding*. doi.org/10.1111/pbr.12782

Lopes, M. S., Reynolds, M. P., McIntyre, C. L., Mathews, K. L., Jalal Kamali, M. R., Mossad, M., Feltaous, Y., Tahir, I. S. A., Chatrath, R., Ogbonnaya, F., & Baum, M. (2013). QTL for yield and associated traits in the Seri/Babax population grown across several environments in Mexico, in the West Asia, North Africa, and South Asia regions. *Theoretical and Applied Genetics*, *126*(4). doi.org/10.1007/s00122-012-2030-4

Loukoianov, A., Yan, L., Blechl, A., Sanchez, A., & Dubcovsky, J. (2005). Regulation of *VRN-1* vernalization genes in normal and transgenic polyploid wheat. *Plant Physiology*, *138*(4), 2364–2373. doi.org/10.1104/pp.105.064287

Lu, Q., Lillemo, M., Skinnes, H., He, X., Shi, J., Ji, F., Dong, Y., & Bjørnstad, Å. (2012). Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line 'Shanghai-3/Catbird.' *Theoretical and Applied Genetics*, *126*(2). doi.org/10.1007/s00122-012-1981-9

Lumpkin, T. A. (2015). How a gene from Japan revolutionized the world of wheat: CIMMYT's quest for combining genes to mitigate threats to global food security. In Y. Ogihara, S. Takumi, & H. Handa (Eds.), *Advances in wheat genetics: From genome to field*. Springer. ISBN: 978-4-431-55674-9

Lyra, D. H., Virlet, N., Sadeghi-Tehran, P., Hassall, K. L., Wingen, L. U., Orford, S., Griffiths, S., Hawkesford, M. J., & Slavov, G. T. (2020). Functional QTL mapping and genomic prediction of canopy height in wheat measured using a robotic field phenotyping platform. *Journal of Experimental Botany*, *71*(6). doi.org/10.1093/jxb/erz545

Ma, J., Ding, P., Liu, J., Li, T., Zou, Y., Habib, A., Mu, Y., Tang, H., Jiang, Q., Liu, Y., Chen, G., Wang, J., Deng, M., Qi, P., Li, W., Pu, Z., Zheng, Y., Wei, Y., & Lan, X. (2019a). Identification and validation of a major and stably expressed QTL for spikelet number per spike in bread wheat. *Theoretical and Applied Genetics*, *132*(11). doi.org/10.1007/s00122-019-03415-z

Ma, J., Zhang, H., Li, S., Zou, Y., Li, T., Liu, J., Ding, P., Mu, Y., Tang, H., Deng, M., Liu, Y., Jiang, Q., Chen, G., Kang, H., Li, W., Pu, Z., Wei, Y., Zheng, Y., & Lan, X. (2019b). Identification of quantitative trait loci for kernel traits in a wheat cultivar Chuannong16. *BMC Genetics*, *20*(1). doi.org/10.1186/s12863-019-0782-4

Mackay, T. F. C., Stone, E. A., & Ayroles, J. F. (2009). The genetics of quantitative traits: Challenges and prospects. *Nature Reviews Genetics*, *10*(8). doi.org/10.1038/nrg2612

Maes, T., De Keukeleire, P., & Gerats, T. (1999). Plant tagnology. *Trends in Plant Science*, *4*(3). doi.org/10.1016/s1360-1385(99)01375-8

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., & Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science*, *339*(6121). doi.org/10.1126/science.1232033

Manly, K. F., Cudmore, Robert H., & Meer, J. M. (2001). Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome*, *12*(12). doi.org/10.1007/s00335-001-1016-3

Mann, G., Diffey, S., Cullis, B., Azanza, F., Martin, D., Kelly, A., McIntyre, L., Schmidt, A., Ma, W., Nath, Z., Kutty, I., Leyne, P. E., Rampling, L., Quail, K. J., & Morell, M. K. (2009). Genetic control of wheat quality: Interactions between chromosomal regions determining protein content and composition, dough rheology, and sponge and dough baking properties. *Theoretical and Applied Genetics*, *118*(8). doi.org/10.1007/s00122-009-1000-y

Maphosa, L., Langridge, P., Taylor, H., Parent, B., Emebiri, L. C., Kuchel, H., Reynolds, M. P., Chalmers, K. J., Okada, A., Edwards, J., & Mather, D. E. (2014). Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions. *Theoretical and Applied Genetics*, *127*(7). doi.org/10.1007/s00122-014-2322-y

Maphosa, L., Langridge, P., Taylor, H., Emebiri, L. C., & Mather, D. E. (2015). Genetic control of grain protein, dough rheology traits and loaf traits in a bread wheat population grown in three environments. *Journal of Cereal Science*, *64*. doi.org/10.1016/j.jcs.2015.05.010

Marino, R., Volante, A., Brandolini, A., & Heun, M. (2018). A high-resolution einkorn (*Triticum monococcum* L.) linkage map involving wild, domesticated and feral einkorn genotypes. *Plant Breeding*, *137*(5). doi.org/10.1111/pbr.12637

Martínez, O., & Curnow, R. N. (1994). Missing markers when estimating quantitative trait loci using regression mapping. *Heredity*, *73*(2). doi.org/10.1038/hdy.1994.120

Marza, F., Bai, G.-H., Carver, B. F., & Zhou, W.-C. (2005). Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. *Theoretical and Applied Genetics*, *112*(4). doi.org/10.1007/s00122-005-0172-3

Maystrenko, O. I. (1976). Identification and location of genes controlling leaf hairing in young plants of common wheat. *Russian Journal of Genetics*, *12*.

McCallum, C. M., Comai, L., Greene, E. A., & Henikoff, S. (2000). Targeting induced local lesions in genomes (TILLING) for plant functional genomics. *Plant Physiology*, *123*(2). doi.org/10.1104/pp.123.2.439

McCartney, C. A., Somers, D. J., Humphreys, D. G., Lukow, O., Ames, N., Noll, J., Cloutier, S., & McCallum, B. D. (2005). Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 × "AC Domain." *Genome*, *48*(5). doi.org/10.1139/g05-055

McFadden, E. S., & Sears, E. R. (1946). The origin of *Triticum spelta* and its free-threshing hexaploid relatives. *Journal of Heredity*, *37*(3). doi.org/10.1093/oxfordjournals.jhered.a105590

Meissner, R., Chague, V., Zhu, Q., Emmanuel, E., Elkind, Y., & Levy, A. A. (2000). A high throughput system for transposon tagging and promoter trapping in tomato. *The Plant Journal*, *22*(3). doi.org/10.1046/j.1365-313x.2000.00735.x

Members of the Complex Trait Consortium (2003). The nature and identification of quantitative trait loci: A community's view. *Nature Reviews Genetics*, *4*(11). doi.org/10.1038/nrg1206

Meng, L., Li, H., Zhang, L., & Wang, J. (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *The Crop Journal*, *3*(3). doi.org/10.1016/j.cj.2015.01.001

Mester, D., Ronin, Y., Minkov, D., Nevo, E., & Korol, A. (2003). Constructing large-scale genetic maps using an evolutionary strategy algorithm. *Genetics*, *165*(4). genetics.org/content/165/4/2269

Mesterházy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant Breeding*, *114*(5). doi.org/10.1111/j.1439-0523.1995.tb00816.x

Michelmore, R. W., Paran, I., & Kesseli, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences*, *88*(21). doi.org/10.1073/pnas.88.21.9828

Morton, N. E. (1955). Sequential tests for the detection of linkage. *American Journal of Human Genetics*, *7*(3). ncbi.nlm.nih.gov/pmc/articles/PMC1716611/

Muqaddasi, Q. H., Brassac, J., Koppolu, R., Plieske, J., Ganal, M. W., & Röder, M. S. (2019). *TaAPO-A1*, an ortholog of rice *ABERRANT PANICLE ORGANIZATION 1*, is associated with total spikelet number per spike in elite European hexaploid winter wheat (*Triticum aestivum* L.) varieties. *Scientific Reports*, *9*(1). doi.org/10.1038/s41598-019-50331-9

Nalam, V. J., Vales, M. I., Watson, C. J. W., Kianian, S. F., & Riera-Lizarazu, O. (2006). Map-based analysis of genes affecting the brittle rachis character in tetraploid wheat (*Triticum turgidum* L.). *Theoretical and Applied Genetics*, *112*(2). doi.org/10.1007/s00122-005-0140-y

Nalam, V. J., Vales, M. I., Watson, C. J. W., Johnson, E. B., & Riera-Lizarazu, O. (2007). Map-based analysis of genetic loci on chromosome 2D that affect glume tenacity and threshability, components of the free-threshing habit in common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *116*(1). doi.org/10.1007/s00122-007-0653-7

Narasimhamoorthy, B., Gill, B. S., Fritz, A. K., Nelson, J. C., & Brown-Guedira, G. L. (2006). Advanced backcross QTL analysis of a hard winter wheat × synthetic wheat population. *Theoretical and Applied Genetics*, *112*(5). doi.org/10.1007/s00122-005-0159-0

Neff, M. M., Neff, J. D., Chory, J., & Pepper, A. E. (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: Experimental applications in *Arabidopsis thaliana* genetics. *The Plant Journal: For Cell and Molecular Biology*, *14*(3). doi.org/10.1046/j.1365-313x.1998.00124.x

Nesbitt, M., & Samuel, D. (1996). From staple crop to extinction? The archaeology and history of the hulled wheats. In S. Padulosi, K. Hammer, & J. Heller (Eds.), *Hulled wheats. Proceedings of the First International Workshop on Hulled Wheats. Promoting the conservation and use of underutilized and neglected crops 4.* International Plant Genetic Resources Institute. ISBN: 92-9043-288-8

Nigro, D., Gadaleta, A., Mangini, G., Colasuonno, P., Marcotuli, I., Giancaspro, A., Giove, S. L., Simeone, R., & Blanco, A. (2019). Candidate genes and genome-wide association study of grain protein content and protein deviation in durum wheat. *Planta*, *249*(4). doi.org/10.1007/s00425-018-03075-1

Niu, Z., Jiang, A., Abu Hammad, W., Oladzadabbasabadi, A., Xu, S. S., Mergoum, M., & Elias, E. M. (2014). Review of doubled haploid production in durum and common wheat through wheat × maize hybridization. *Plant Breeding*, *133*(3). doi.org/10.1111/pbr.12162

Norris, D. M., & Kogan, M. (1980). Biochemical and morphological bases of resistance. In F. G. Maxwell & P. R. Jennings (Eds.), *Breeding plants resistant to insects*. Wiley. ISBN: 0-471-03268-9

Ochagavía, H., Prieto, P., Savin, R., Griffiths, S., & Slafer, G. A. (2017). Duration of developmental phases, and dynamics of leaf appearance and tillering, as affected by source and doses of photoperiod insensitivity alleles in wheat under field conditions. *Field Crops Research*, *214*. doi.org/10.1016/j.fcr.2017.08.015

Olson, M., Hood, L., Cantor, C., & Botstein, D. (1989). A common language for physical mapping of the human genome. *Science*, *245*(4925). doi.org/10.1126/science.2781285

Özkan, H., Willcox, G., Graner, A., Salamini, F., & Kilian, B. (2010). Geographic distribution and domestication of wild emmer wheat (*Triticum dicoccoides*). *Genetic Resources and Crop Evolution*, *58*(1). doi.org/10.1007/s10722-010-9581-5

Peleg, Z., Cakmak, I., Ozturk, L., Yazici, A., Jun, Y., Budak, H., Korol, A. B., Fahima, T., & Saranga, Y. (2009). Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theoretical and Applied Genetics*, *119*(2). doi.org/10.1007/s00122-009-1044-z

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F., Sudhakar, D., Christou, P., Snape, J. W., Gale, M. D., & Harberd, N. P. (1999). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature*, *400*(6741). doi.org/10.1038/22307

Peng, J., Ronin, Y., Fahima, T., Roder, M. S., Li, Y., Nevo, E., & Korol, A. (2003). Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. *Proceedings of the National Academy of Sciences*, *100*(5). doi.org/10.1073/pnas.252763199

Pflieger, S., Lefebvre, V., & Causse, M. (2001). The candidate gene approach in plant genetics: A review. *Molecular Breeding*, *7*(4). doi.org/10.1023/a:1011605013259

Pickering, R. A., Hill, A. M., & Kynast, R. G. (1997). Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum vulgare* L. × *Hordeum bulbosum* L. crosses. *Genome*, *40*(2). doi.org/10.1139/g97-028

Pont, C., Leroy, T., Seidel, M., Tondelli, A., Duchemin, W., Armisen, D., Lang, D., Bustos-Korts, D., Goué, N., Balfourier, F., Molnár-Láng, M., Lage, J., Kilian, B., Özkan, H., Waite, D., Dyer, S., Letellier, T., Alaux, M., Russell, J., … Salse, J. (2019). Tracing the ancestry of modern bread wheats. *Nature Genetics*, *51*(5). doi.org/10.1038/s41588-019-0393-z

Pourkheirandish, M., Dai, F., Sakuma, S., Kanamori, H., Distelfeld, A., Willcox, G., Kawahara, T., Matsumoto, T., Kilian, B., & Komatsuda, T. (2018). On the origin of the non-brittle rachis trait of domesticated einkorn wheat. *Frontiers in Plant Science*, *8*(2031). doi.org/10.3389/fpls.2017.02031

Prashant, R., Kadoo, N., Desale, C., Kore, P., Dhaliwal, H. S., Chhuneja, P., & Gupta, V. (2012). Kernel morphometric traits in hexaploid wheat (*Triticum aestivum* L.) are modulated by intricate QTL × QTL and genotype × environment interactions. *Journal of Cereal Science*, *56*(2). doi.org/10.1016/j.jcs.2012.05.010

Pshenichnikova, T. A., Lapochkina, I. F., & Shchukina, L. V. (2006). The inheritance of morphological and biochemical traits introgressed into common wheat (*Triticum aestivum* L.) from Aegilops speltoides Tausch. *Genetic Resources and Crop Evolution*, *54*(2). doi.org/10.1007/s10722-005-4499-z

Pshenichnikova, T. A., Doroshkov, A. V., Simonov, A. V., Afonnikov, D. A., & Börner, A. (2016). Diversity of leaf pubescence in bread wheat and relative species. *Genetic Resources and Crop Evolution*, *64*(7). doi.org/10.1007/s10722-016-0471-3

Pshenichnikova, T. A., Doroshkov, A. V., Osipova, S. V., Permyakov, A. V., Permyakova, M. D., Efimov, V. M., & Afonnikov, D. A. (2018). Quantitative characteristics of pubescence in wheat (*Triticum aestivum* L.) are associated with photosynthetic parameters under conditions of normal and limited water supply. *Planta*, *249*(3). doi.org/10.1007/s00425-018-3049-9

Quarrie, S. A., Steed, A., Calestani, C., Semikhodskii, A., Lebreton, C., Chinoy, C., Steele, N., Pljevljakusić, D., Waterman, E., Weyen, J., Schondelmaier, J., Habash, D. Z., Farmer, P., Saker, L., Clarkson, D. T., Abugalieva, A., Yessimbekova, M., Turuspekov, Y., Abugalieva, S., … Dodig, D. (2005). A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theoretical and Applied Genetics*, *110*(5). doi.org/10.1007/s00122-004-1902-7

Rabinovich, S. V. (1998). Importance of wheat-rye translocations for breeding modern cultivar of *Triticum aestivum* L. Euphytica, 100(1/3), 323–340. doi.org/10.1023/a:1018361819215

Reif, J. C., Gowda, M., Maurer, H. P., Longin, C. F. H., Korzun, V., Ebmeyer, E., Bothe, R., Pietsch, C., & Würschum, T. (2011). Association mapping for quality traits in soft winter wheat. *Theoretical and Applied Genetics*, *122*(5). doi.org/10.1007/s00122-010-1502-7

Ren, T., Hu, Y., Tang, Y., Li, C., Yan, B., Ren, Z., Tan, F., Tang, Z., Fu, S., & Li, Z. (2018). Utilization of a Wheat55K SNP array for mapping of major QTL for temporal expression of the tiller number. *Frontiers in Plant Science*, *9*(333). doi.org/10.3389/fpls.2018.00333

Richards, R. (1988). A tiller inhibitor gene in wheat and its effect on plant growth. *Australian Journal of Agricultural Research*, *39*(5). doi.org/10.1071/ar9880749

Riley, R., Unrau, J., & Chapman, V. (1958). Evidence on the origin of the B genome of wheat. *Journal of Heredity*, *49*(3). doi.org/10.1093/oxfordjournals.jhered.a106784

Roberts, J. J., Gallun, R. L., Patterson, F. L., & Foster, J. E. (1979). Effects of wheat leaf pubescence on the hessian fly. *Journal of Economic Entomology*, *72*(2). doi.org/10.1093/jee/72.2.211

Ronin, Y. I., Mester, D. I., Minkov, D. G., Akhunov, E., & Korol, A. B. (2017). Building ultra-high-density linkage maps based on efficient filtering of trustable markers. *Genetics*, *206*(3). doi.org/10.1534/genetics.116.197491

Saade, S., Kutlu, B., Draba, V., Förster, K., Schumann, E., Tester, M., Pillen, K., & Maurer, A. (2017). A donor-specific QTL, exhibiting allelic variation for leaf sheath hairiness in a nested association mapping population, is located on barley chromosome 4H. *PLoS One*, *12*(12). doi.org/10.1371/journal.pone.0189446

Sakuma, S., Golan, G., Guo, Z., Ogawa, T., Tagiri, A., Sugimoto, K., Bernhardt, N., Brassac, J., Mascher, M., Hensel, G., Ohnishi, S., Jinno, H., Yamashita, Y., Ayalon, I., Peleg, Z., Schnurbusch, T., & Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences*, *116*(11). doi.org/10.1073/pnas.1815465116

Salina, E., Börner, A., Leonova, I., Korzun, V., Laikova, L., Maystrenko, O., & Röder, M. S. (2000). Microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*. *Theoretical and Applied Genetics*, *100*(5). doi.org/10.1007/s001220051340

Sando, W. J. (1935). Hybrids of wheat, rye, *Aegilops* and *Haynaldia*: A series of 122 intra- and inter-generic hybrids shows wide variations in fertility. Journal of Heredity, 26(6), 229–232. https://doi.org/10.1093/oxfordjournals.jhered.a104084

Sarkar, P., & Stebbins, G. L. (1956). Morphological evidence concerning the origin of the B genome in wheat. *American Journal of Botany*, *43*(4). doi.org/10.1002/j.1537-2197.1956.tb10494.x

Sarret, G., Harada, E., Choi, Y.-E., Isaure, M.-P., Geoffroy, N., Fakra, S., Marcus, M. A., Birschwilks, M., Clemens, S., & Manceau, A. (2006). Trichomes of tobacco excrete zinc as zinc-substituted calcium carbonate and other zinc-containing compounds. *Plant Physiology*, *141*(3). doi.org/10.1104/pp.106.082743

Schiemann, E. (1948). *Weizen, Roggen, Gerste: Systematik, Geschichte und Verwendung. Mit 25 Figuren im Text und einer Stammtafel des Weizens*. Gustav Fischer.

Schillinger, J. A., & Gallun, R. L. (1968). Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, *Oulema melanopus*. *Annals of the Entomological Society of America*, *61*(4). doi.org/10.1093/aesa/61.4.900

Schilmiller, A. L., Last, R. L., & Pichersky, E. (2008). Harnessing plant trichome biochemistry for the production of useful compounds. *The Plant Journal*, *54*(4). doi.org/10.1111/j.1365-313x.2008.03432.x

Schneider, K. (2005). Mapping populations and principles of genetic mapping. In K. Meksem & G. Kahl (Eds.), *The handbook of plant genome mapping: Genetic and physical mapping*. Wiley. ISBN: 978-352-7311-163

Searchinger, T., Waite, R., Hanson, C., Ranganathan, J., & Matthews, E. (2019). *World Resources Report: Creating a sustainable food future*. World Resources Institute. wrr-food.wri.org/

Sehgal, D., Singh, R., & Rajpal, V. R. (2016). Quantitative trait loci mapping in plants: Concepts and approaches. In V. R. Rajpal, S. Rama Rao, & S. N. Raina (Eds.), *Molecular breeding for sustainable crop improvement, volume 2*. Springer. ISBN: 978-3-319-27090-6

Sharma, H. C., Foster, J. E., Ohm, H. W., & Patterson, F. L. (1992). A note on resistance to Hessian fly (*Mayetiola destructor*) [*Diptera*: *Cecidomyidae*] biotype L in tribe *Triticeae*. *Phytoprotection*, *73*(2). doi.org/10.7202/706023ar

Sharma, H. C., & Waines, J. G. (1994). Inheritance of leaf pubescence in diploid wheat. *Journal of Heredity*, *85*(4). doi.org/10.1093/oxfordjournals.jhered.a111457

Simmons, A. T., Gurr, G. M., McGrath, D., Martin, P. M., & Nicol, H. I. (2004). Entrapment of *Helicoverpa armigera* (Hübner) (*Lepidoptera*: *Noctuidae*) on glandular trichomes of *Lycopersicon* species. *Australian Journal of Entomology*, *43*(2). doi.org/10.1111/j.1440-6055.2004.00414.x

Simonetti, M. C., Bellomo, M. P., Laghetti, G., Perrino, P., Simeone, R., & Blanco, A. (1999). Quantitative trait loci influencing free-threshing habit in tetraploid wheats. *Genetic Resources and Crop Evolution*, *46*(3). doi.org/10.1023/a:1008602009133

Simons, K. J., Fellers, J. P., Trick, H. N., Zhang, Z., Tai, Y.-S., Gill, B. S., & Faris, J. D. (2006). Molecular characterization of the major wheat domestication gene *Q*. *Genetics*, *172*(1). doi.org/10.1534/genetics.105.044727

Singh, K., Ghai, M., Garg, M., Chhuneja, P., Kaur, P., Schnurbusch, T., Keller, B., & Dhaliwal, H. S. (2007). An integrated molecular linkage map of diploid wheat based on a *Triticum boeoticum* × *T. monococcum* RIL population. *Theoretical and Applied Genetics*, *115*(3). doi.org/10.1007/s00122-007-0543-z

Singh, K, Chhuneja, P., Kaur, S., Garg, T., Tiwari, V. K., Rawat, N., Bains, N. S., Dhaliwal, H. S., & Keller, B. (2008). *Triticum monococcum*: A source of novel genes for improving several traits in hexaploid wheat. In R. Appels, R. Eastwood, E. Lagudah, P. Langridge, & M. Mackay Lynne (Eds.), *Proceedings of the 11th International Wheat Genetics Symposium*. Sydney University Press. http://hdl.handle.net/2123/3430

Slafer, G. A. (2003). Genetic basis of yield as viewed from a crop physiologist's perspective. *Annals of Applied Biology*, *142*(2). doi.org/10.1111/j.1744-7348.2003.tb00237.x

Slatkin, M. (1999). Disequilibrium mapping of a quantitative-trait locus in an expanding population. *The American Journal of Human Genetics*, *64*(6). doi.org/10.1086/302413

Slykhuis, J. T. (1955). Aceria tulipae Keifer (Acarina: Eriophyidae) in relation to the spread of wheat streak mosaic. *Phytopathology*, *45*(3).

Solovyev, V., Kosarev, P., Seledsov, I., & Vorobyev, D. (2006). Automatic annotation of eukaryotic genes, pseudogenes and promoters. *Genome Biology*, *7*(Suppl 1). doi.org/10.1186/gb-2006-7-s1-s10

Sood, S., Kuraparthy, V., Bai, G., & Gill, B. S. (2009). The major threshability genes soft glume (*sog*) and tenacious glume (*Tg*), of diploid and polyploid wheat, trace their origin to independent mutations at non-orthologous loci. *Theoretical and Applied Genetics*, *119*(2). doi.org/10.1007/s00122-009-1043-0

Sourdille, P., Cadalen, T., Gay, G., Gill, B., & Bernard, M. (2002). Molecular and physical mapping of genes affecting awning in wheat. *Plant Breeding*, *121*(4). doi.org/10.1046/j.1439-0523.2002.728336.x

Sourdille, P., Cadalen, T., Guyomarc'h, H., Snape, J., Perretant, M., Charmet, G., Boeuf, C., Bernard, S., & Bernard, M. (2003). An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. *Theoretical and Applied Genetics*, *106*(3). doi.org/10.1007/s00122-002-1044-8

Spielmeyer, W., & Richards, R. A. (2004). Comparative mapping of wheat chromosome 1AS which contains the tiller inhibition gene (*tin*) with rice chromosome 5S. *Theoretical and Applied Genetics*, *109*(6). doi.org/10.1007/s00122-004-1745-2

Stam, P. (1993). Construction of integrated genetic linkage maps by means of a new computer package: Join Map. *The Plant Journal*, *3*(5). doi.org/10.1111/j.1365-313x.1993.00739.x

Sturtevant, A. H. (1913). The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. *Journal of Experimental Zoology*, *14*(1). doi.org/10.1002/jez.1400140104

Su, Z., Hao, C., Wang, L., Dong, Y., & Zhang, X. (2011). Identification and development of a functional marker of *TaGW2* associated with grain weight in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *122*(1). doi.org/10.1007/s00122-010-1437-z

Su, Q., Zhang, X., Zhang, W., Zhang, N., Song, L., Liu, L., Xue, X., Liu, G., Liu, J., Meng, D., Zhi, L., Ji, J., Zhao, X., Yang, C., Tong, Y., Liu, Z., & Li, J. (2018). QTL detection for kernel size and weight in bread wheat (*Triticum aestivum* L.) using a high-density SNP and SSR-based linkage map. *Frontiers in Plant Science*, *9*(1484). doi.org/10.3389/fpls.2018.01484

Taenzler, B., Esposti, R. F., Vaccino, P., Brandolini, A., Effgen, S., Heun, M., Schäfer-Pregl, R., Borghi, B., & Salamini, F. (2002). Molecular linkage map of Einkorn wheat: Mapping of storage-protein and soft-glume genes and bread-making quality QTLs. *Genetical Research*, *80*(2). doi.org/10.1017/s001667230200575x

Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., Uemura, A., Utsushi, H., Tamiru, M., Takuno, S., Innan, H., Cano, L. M., Kamoun, S., & Terauchi, R. (2013). QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal*, *74*(1). doi.org/10.1111/tpj.12105

Takahashi, R., & Hayashi, J. (1966). Inheritance and linkage studies in barley. II. Assignment of several new mutant genes to their respective linkage groups by the trisomic method of analysis. *Berichte Des Ohara Instituts Für Landwirtschaftliche Biologie*, *13*.

Taketa, S., Chang, C. L., Ishii, M., & Takeda, K. (2002). Chromosome arm location of the gene controlling leaf pubescence of a Chinese local wheat cultivar 'Hong-mang-mai.' *Euphytica*, *125*(2). doi.org/10.1023/a:1015812907111

Tanno, K., & Willcox, G. (2006). How fast was wild wheat domesticated? *Science*, *311*(5769). doi.org/10.1126/science.1124635

Tattini, M., Gravano, E., Pinelli, P., Mulinacci, N., & Romani, A. (2000). Flavonoids accumulate in leaves and glandular trichomes of *Phillyrea latifolia* exposed to excess solar radiation. *New Phytologist*, *148*(1). doi.org/10.1046/j.1469-8137.2000.00743.x

Tian, J., Deng, Z., Zhang, K., Yu, H., Jiang, X., & Li, C. (2015). *Genetic analyses of wheat and molecular marker-assisted breeding, volume 1*. Springer. doi.org/10.1007/978-94-017-7390-4

Tian, X., Wen, W., Xie, L., Fu, L., Xu, D., Fu, C., Wang, D., Chen, X., Xia, X., Chen, Q., He, Z., & Cao, S. (2017). Molecular mapping of reduced plant height gene *Rht24* in bread wheat. *Frontiers in Plant Science*, *8*(1379). doi.org/10.3389/fpls.2017.01379

Tiwari, V. K., Faris, J. D., Friebe, B., & Gill, B. S. (2016a). Genome mapping. In C. Wrigley, H. Corke, K. Seetharaman, & J. Faubion (Eds.), *Encyclopedia of Food Grains*. Academic Press. ISBN: 978-0-12-394786-4

Tiwari, C., Wallwork, H., Arun, B., Mishra, V. K., Velu, G., Stangoulis, J., Kumar, U., & Joshi, A. K. (2016b). Molecular mapping of quantitative trait loci for zinc, iron and protein content in the grains of hexaploid wheat. *Euphytica*, *207*(3). doi.org/10.1007/s10681-015-1544-7

Tsilo, T. J., Hareland, G. A., Simsek, S., Chao, S., & Anderson, J. A. (2010). Genome mapping of kernel characteristics in hard red spring wheat breeding lines. *Theoretical and Applied Genetics*, *121*(4). doi.org/10.1007/s00122-010-1343-4

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., & Webb, D. A. (1980). *Flora Europaea, Vol. 5,* Alismataceae *to* Orchidaceae (Monocotyledones) (D. A. Webb, Ed.; 7th ed.). Cambridge University Press. ISBN: 9780521201087

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15). doi.org/10.1093/nar/gks596

Valárik, M., Linkiewicz, A. M., & Dubcovsky, J. (2006). A microcolinearity study at the earliness *per se* gene *Eps-A^{m}1* region reveals an ancient duplication that preceded the wheat–rice divergence. *Theoretical and Applied Genetics*, *112*(5). doi.org/10.1007/s00122-005-0198-6

Van Boxstael, F., Aerts, H., Linssen, S., Latré, J., Christiaens, A., Haesaert, G., Dierickx, I., Brusselle, J., & De Keyzer, W. (2020). A comparison of the nutritional value of Einkorn, Emmer, Khorasan and modern wheat: Whole grains, processed in bread, and population-level intake implications. *Journal of the Science of Food and Agriculture*. doi.org/10.1002/jsfa.10402

Vanžurová, H. (2013). *Charakterizace diploidní pšenice* Triticum monococcum *jako zdroje nových genů pro šlechtění pšenice*. Přírodovědecká fakulta Univerzity Palackého. theses.cz/id/ll1myv

Vogel, K. P., Johnson, V. A., & Mattern, P. J. (1976). Protein and lysine content of grain, endosperm, and bran of wheats from the USDA World Wheat Collection. *Crop Science*, *16*(5). doi.org/10.2135/cropsci1976.0011183x001600050014x

Voirin, B., Bayet, C., & Colson, M. (1993). Demonstration that flavone aglycones accumulate in the peltate glands of *Mentha* x *piperita* leaves. *Phytochemistry*, *34*(1). doi.org/10.1016/s0031-9422(00)90787-8

Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1). doi.org/10.1093/jhered/93.1.77

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., & Kuiper, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*(21). ncbi.nlm.nih.gov/pmc/articles/PMC307397/

Wan, H., Yang, Y., Li, J., Zhang, Z., & Yang, W. (2015). Mapping a major QTL for hairy leaf sheath introgressed from *Aegilops tauschii* and its association with enhanced grain yield in bread wheat. *Euphytica*, *205*(1). doi.org/10.1007/s10681-015-1457-5

Wang, R. X., Hai, L., Zhang, X. Y., You, G. X., Yan, C. S., & Xiao, S. H. (2009). QTL mapping for grain filling rate and yield-related traits in RILs of the Chinese winter wheat population Heshangmai × Yu8679. *Theoretical and Applied Genetics*, *118*(2). doi.org/10.1007/s00122-008-0901-5

Wang, J., Liu, W., Wang, H., Li, L., Wu, J., Yang, X., Li, X., & Gao, A. (2010). QTL mapping of yield-related traits in the wheat germplasm 3228. *Euphytica*, *177*(2). doi.org/10.1007/s10681-010-0267-z

Wang, S, Basten, C. J., & Zeng, Z.-B. (2012a). *Windows QTL Cartographer 2.5*. Department of Statistics, North Carolina State University. brcwebportal.cos.ncsu.edu/qtlcart/WQTLCart.htm

Wang, L., Cui, F., Wang, J., Jun, L., Ding, A., Zhao, C., Li, X., Feng, D., Gao, J., & Wang, H. (2012b). Conditional QTL mapping of protein content in wheat with respect to grain yield and its components. *Journal of Genetics*, *91*(3). doi.org/10.1007/s12041-012-0190-2

Wang, J., Luo, M.-C., Chen, Z., You, F. M., Wei, Y., Zheng, Y., & Dvořák, J. (2013). *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. *New Phytologist*, *198*(3). doi.org/10.1111/nph.12164

Wang, J., Yu, H., Weng, X., Xie, W., Xu, C., Li, X., Xiao, J., & Zhang, Q. (2014). An expression quantitative trait loci-guided co-expression analysis for constructing regulatory network using a rice recombinant inbred line population. *Journal of Experimental Botany*, *65*(4). doi.org/10.1093/jxb/ert464

Wang, Z., Liu, Y., Shi, H., Mo, H., Wu, F., Lin, Y., Gao, S., Wang, J., Wei, Y., Liu, C., & Zheng, Y. (2016a). Identification and validation of novel low-tiller number QTL in common wheat. *Theoretical and Applied Genetics*, *129*(3). doi.org/10.1007/s00122-015-2652-4

Wang, S., Yan, X., Wang, Y., Liu, H., Cui, D., & Chen, F. (2016b). Haplotypes of the *TaGS5-A1* gene are associated with thousand-kernel weight in Chinese bread wheat. *Frontiers in Plant Science*, *7*(783). doi.org/10.3389/fpls.2016.00783

Wang, X., Dong, L., Hu, J., Pang, Y., Hu, L., Xiao, G., Ma, X., Kong, X., Jia, J., Wang, H., & Kong, L. (2019). Dissecting genetic loci affecting grain morphological traits to improve grain weight via nested association mapping. *Theoretical and Applied Genetics*, *132*(11). doi.org/10.1007/s00122-019-03410-4

Watanabe, N., & Ikebata, N. (2000). The effects of homoeologous group 3 chromosomes on grain colour dependent seed dormancy and brittle rachis in tetraploid wheat. *Euphytica*, *115*(3). doi.org/10.1023/a:1004066416900

Watanabe, N., Sugiyama, K., Yamagishi, Y., & Sakata, Y. (2003). Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. *Hereditas*, *137*(3). doi.org/10.1034/j.1601-5223.2002.01609.x

Waterhouse, P. M., Graham, M. W., & Wang, M. B. (1998). Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(23). doi.org/10.1073/pnas.95.23.13959

Waterhouse, P. M., & Helliwell, C. A. (2003). Exploring plant genomes by RNA-induced gene silencing. *Nature Reviews Genetics*, *4*(1). doi.org/10.1038/nrg982

Webster, J. A. (1975). *Association of plant hairs and insect resistance: An annotated bibliography. Miscellaneous Publication No. 1297*. United States Department of Agriculture.

Weinhold, A., & Baldwin, I. T. (2011). Trichome-derived *o*-acyl sugars are a first meal for caterpillars that tags them for predation. *Proceedings of the National Academy of Sciences*, *108*(19). doi.org/10.1073/pnas.1101306108

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, *18*(22). doi.org/10.1093/nar/18.22.6531

Williams, K., & Sorrells, M. E. (2014). Three-dimensional seed size and shape QTL in hexaploid wheat (*Triticum aestivum* L.) populations. *Crop Science*, *54*(1). doi.org/10.2135/cropsci2012.10.0609

Wu, R., & Zeng, Z.-B. (2001). Joint linkage and linkage disequilibrium mapping in natural populations. *Genetics*, *157*(2). genetics.org/content/157/2/899.full

Wu, R., Ma, C., & Casella, G. (2002). Joint linkage and linkage disequilibrium mapping of quantitative trait loci in natural populations. *Genetics*, *160*(2). ncbi.nlm.nih.gov/pmc/articles/PMC1461972/

Wu, X., Chang, X., & Jing, R. (2012). Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. *PLoS ONE*, *7*(2). doi.org/10.1371/journal.pone.0031249

Wu, Q.-H., Chen, Y.-X., Zhou, S.-H., Fu, L., Chen, J.-J., Xiao, Y., Zhang, D., Ouyang, S.-H., Zhao, X.-J., Cui, Y., Zhang, D.-Y., Liang, Y., Wang, Z.-Z., Xie, J.-Z., Qin, J.-X., Wang, G.-X., Li, D.-L., Huang, Y.-L., Yu, M.-H., … Liu, Z.-Y. (2015). High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817 × Beinong6. *PLOS ONE*, *10*(2). doi.org/10.1371/journal.pone.0118144

Würschum, T., Langer, S. M., Longin, C. F. H., Tucker, M. R., & Leiser, W. L. (2017). A modern Green Revolution gene for reduced height in wheat. *The Plant Journal*, *92*(5). doi.org/10.1111/tpj.13726

Würschum, Tobias, Leiser, W. L., Langer, S. M., Tucker, M. R., & Longin, C. F. H. (2018). Phenotypic and genetic analysis of spike and kernel characteristics in wheat reveals long-term genetic trends of grain yield components. *Theoretical and Applied Genetics*, *131*(10). doi.org/10.1007/s00122-018-3133-3

Xie, Q., Mayes, S., & Sparkes, D. L. (2015). Optimizing tiller production and survival for grain yield improvement in a bread wheat × spelt mapping population. *Annals of Botany*, *117*(1). doi.org/10.1093/aob/mcv147

Xin, F., Zhu, T., Wei, S., Han, Y., Zhao, Y., Zhang, D., Ma, L., & Ding, Q. (2020). QTL mapping of kernel traits and validation of a major QTL for kernel length-width ratio using SNP and bulked segregant analysis in wheat. *Scientific Reports*, *10*(1). doi.org/10.1038/s41598-019-56979-7

Xu, Y., Wang, R., Tong, Y., Zhao, H., Xie, Q., Liu, D., Zhang, A., Li, B., Xu, H., & An, D. (2013). Mapping QTLs for yield and nitrogen-related traits in wheat: Influence of nitrogen and phosphorus fertilization on QTL expression. *Theoretical and Applied Genetics*, *127*(1). doi.org/10.1007/s00122-013-2201-y

Xu, T., Bian, N., Wen, M., Xiao, J., Yuan, C., Cao, A., Zhang, S., Wang, X., & Wang, H. (2016). Characterization of a common wheat (*Triticum aestivum* L.) high-tillering dwarf mutant. *Theoretical and Applied Genetics*, *130*(3). doi.org/10.1007/s00122-016-2828-6

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., & Dubcovsky, J. (2003). Positional cloning of the wheat vernalization gene *VRN1*. *Proceedings of the National Academy of Sciences*, *100*(10). doi.org/10.1073/pnas.0937399100

Yan, L., Loukoianov, A., Blechl, A., Tranquilli, G., Ramakrishna, W., SanMiguel, P., Bennetzen, J. L., Echenique, V., & Dubcovsky, J. (2004). The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science*, *303*(5664). doi.org/10.1126/science.1094305

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valárik, M., Yasuda, S., & Dubcovsky, J. (2006). The wheat and barley vernalization gene *VRN3* is an orthologue of *FT*. *Proceedings of the National Academy of Sciences*, *103*(51). doi.org/10.1073/pnas.0607142103

Yan, X., Zhao, L., Ren, Y., Dong, Z., Cui, D., & Chen, F. (2019). Genome-wide association study revealed that the *TaGW8* gene was associated with kernel size in Chinese bread wheat. *Scientific Reports*, *9*(1). doi.org/10.1038/s41598-019-38570-2

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2005). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, *38*(2). doi.org/10.1038/ng1702

Yu, J., Holland, J. B., McMullen, M. D., & Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics*, *178*(1). doi.org/10.1534/genetics.107.074245

Yu, K., Liu, D., Wu, W., Yang, W., Sun, J., Li, X., Zhan, K., Cui, D., Ling, H., Liu, C., & Zhang, A. (2016). Development of an integrated linkage map of einkorn wheat and its application for QTL mapping and genome sequence anchoring. *Theoretical and Applied Genetics*, *130*(1). doi.org/10.1007/s00122-016-2791-2

Yu, K., Liu, D., Chen, Y., Wang, D., Yang, W., Yang, W., Yin, L., Zhang, C., Zhao, S., Sun, J., Liu, C., & Zhang, A. (2019). Unraveling the genetic architecture of grain size in einkorn wheat through linkage and homology mapping and transcriptomic profiling. *Journal of Experimental Botany*, *70*(18). doi.org/10.1093/jxb/erz247

Zaharieva, M., & Monneveux, P. (2014). Cultivated einkorn wheat (*Triticum monococcum* L. ssp. *monococcum*): The long life of a founder crop of agriculture. *Genetic Resources and Crop Evolution*, *61*(3). doi.org/10.1007/s10722-014-0084-7

Zanetti, S., Winzeler, M., Feuillet, C., Keller, B., & Messmer, M. (2001). Genetic analysis of bread-making quality in wheat and spelt. *Plant Breeding*, *120*(1). doi.org/10.1046/j.1439-0523.2001.00552.x

Zanke, C. D., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., Argillier, O., Stiewe, G., Hinze, M., Neumann, F., Eichhorn, A., Polley, A., Jaenecke, C., Ganal, M. W., & Röder, M. S. (2015). Analysis of main effect QTL for thousand grain weight in European winter wheat (*Triticum aestivum* L.) by genome-wide association mapping. *Frontiers in Plant Science*, *6*(644). doi.org/10.3389/fpls.2015.00644

Zeng, Z.-B. (1994). Precision mapping of quantitative trait loci. *Genetics*, *136*(4). ncbi.nlm.nih.gov/pmc/articles/PMC1205924/

Zeng, Z.-B., Kao, C. H., & Basten, C. J. (1999). Estimating the genetic architecture of quantitative traits. *Genetical Research*, *74*(3). doi.org/10.1017/s0016672399004255

Zhai, H., Feng, Z., Li, J., Liu, X., Xiao, S., Ni, Z., & Sun, Q. (2016). QTL analysis of spike morphological traits and plant height in winter wheat (*Triticum aestivum* L.) using a high-density SNP and SSR-based linkage map. *Frontiers in Plant Science*, *7*(1617). doi.org/10.3389/fpls.2016.01617

Zhang, J., Dell, B., Biddulph, B., Drake-Brockman, F., Walker, E., Khan, N., Wong, D., Hayden, M., & Appels, R. (2013). Wild-type alleles of *Rht-B1* and *Rht-D1* as independent determinants of thousand-grain weight and kernel number per spike in wheat. *Molecular Breeding*, *32*(4). doi.org/10.1007/s11032-013-9905-1

Zhang, H., Chen, J., Li, R., Deng, Z., Zhang, K., Liu, B., & Tian, J. (2016). Conditional QTL mapping of three yield components in common wheat (*Triticum aestivum* L.). *The Crop Journal*, *4*(3). doi.org/10.1016/j.cj.2016.01.007

Zhang, W., Zhang, M., Zhu, X., Cao, Y., Sun, Q., Ma, G., Chao, S., Yan, C., Xu, S. S., & Cai, X. (2017a). Molecular cytogenetic and genomic analyses reveal new insights into the origin of the wheat B genome. *Theoretical and Applied Genetics*, *131*(2). doi.org/10.1007/s00122-017-3007-0

Zhang, N., Fan, X., Cui, F., Zhao, C., Zhang, W., Zhao, X., Yang, L., Pan, R., Chen, M., Han, J., Ji, J., Liu, D., Zhao, Z., Tong, Y., Zhang, A., Wang, T., & Li, J. (2017b). Characterization of the temporal and spatial expression of wheat (*Triticum aestivum* L.) plant height at the QTL level and their influence on yield-related traits. *Theoretical and Applied Genetics*, *130*(6). doi.org/10.1007/s00122-017-2884-6

Zhang, J., Gizaw, S. A., Bossolini, E., Hegarty, J., Howell, T., Carter, A. H., Akhunov, E., & Dubcovsky, J. (2018). Identification and validation of QTL for grain yield and plant water status under contrasting water treatments in fall-sown spring wheats. *Theoretical and Applied Genetics*, *131*(8). doi.org/10.1007/s00122-018-3111-9

Zhao, C., Zhang, N., Wu, Y., Sun, H., Liu, C., Fan, X., Yan, X., Xu, H., Ji, J., & Cui, F. (2019). QTL for spike-layer uniformity and their influence on yield-related traits in wheat. *BMC Genetics*, *20*(1). doi.org/10.1186/s12863-019-0730-3

Zheng, B. S., Le Gouis, J., Leflon, M., Rong, W. Y., Laperche, A., & Brancourt-Hulmel, M. (2010). Using probe genotypes to dissect QTL × environment interactions for grain yield components in winter wheat. *Theoretical and Applied Genetics*, *121*(8). doi.org/10.1007/s00122-010-1406-6

Zhou, Y., Conway, B., Miller, D., Marshall, D., Cooper, A., Murphy, P., Chao, S., Brown-Guedira, G., & Costa, J. (2017). Quantitative trait loci mapping for spike characteristics in hexaploid wheat. *The Plant Genome*, *10*(2). doi.org/10.3835/plantgenome2016.10.0101

Zikhali, M., Wingen, L. U., Leverington-Waite, M., Specel, S., & Griffiths, S. (2017). The identification of new candidate genes *Triticum aestivum FLOWERING LOCUS T3-B1* (*TaFT3-B1*) and *TARGET OF EAT1* (*TaTOE1-B1*) controlling the short-day photoperiod response in bread wheat. *Plant, Cell & Environment*, *40*(11). doi.org/10.1111/pce.13018

Zohary, D., & Feldman, M. (1962). Hybridization between amphidiploids and the evolution of polyploid in the wheat (*Aegilops-Triticum*) group. *Evolution*, *16*(1). doi.org/10.2307/2406265

Zohary, D., Hopf, M., & Weiss, E. (2013). *Domestication of plants in the Old World: The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley* (4th ed.). Oxford Univ. Press. ISBN: 978-0199688173

9 Appendices

Appendix 1. A linkage map of Triticum monococcum DV92×G3116 with 129 mapped QTLs.

Appendix 2. QTLs detected by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116.

Appendix 3. Highly significant QTLs for leaf pubescence mapped on $3A^m$ and $5A^m$ by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116 using skeleton linkage map.

Appendix 4. Highly significant QTLs for leaf pubescence mapped on $3A^m$ and $5A^m$ by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116 using adjusted global linkage map.

Appendix 5. Verification of the QTLs for leaf pubescence on $3A^m$ and $5A^m$ by single environment multiple interval mapping in *Triticum monococcum* F_2 verification mapping populations.

Appendix 6. *Owm400–Owm448* PCR markers.

Appendix 7. *Owm449–Owm484* PCR markers.

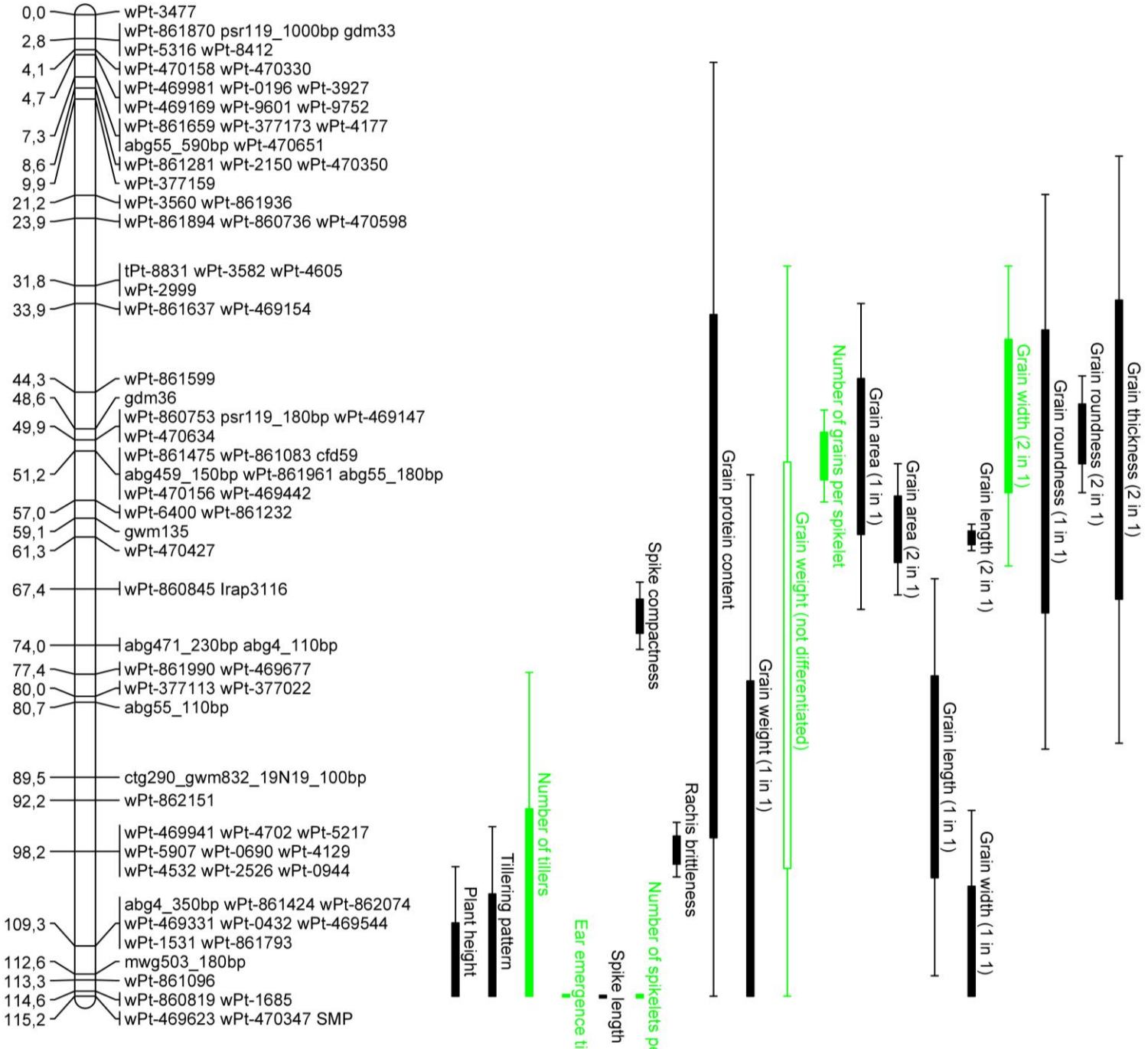
Appendix 1. A linkage map of *Triticum monococcum* DV92×G3116 with 129 mapped QTLs.

The vertical bars represent the seven chromosomes of *T. monococcum* with marker loci on the right and corresponding genetic distances in cM (approximated using Kosambi) on the left.

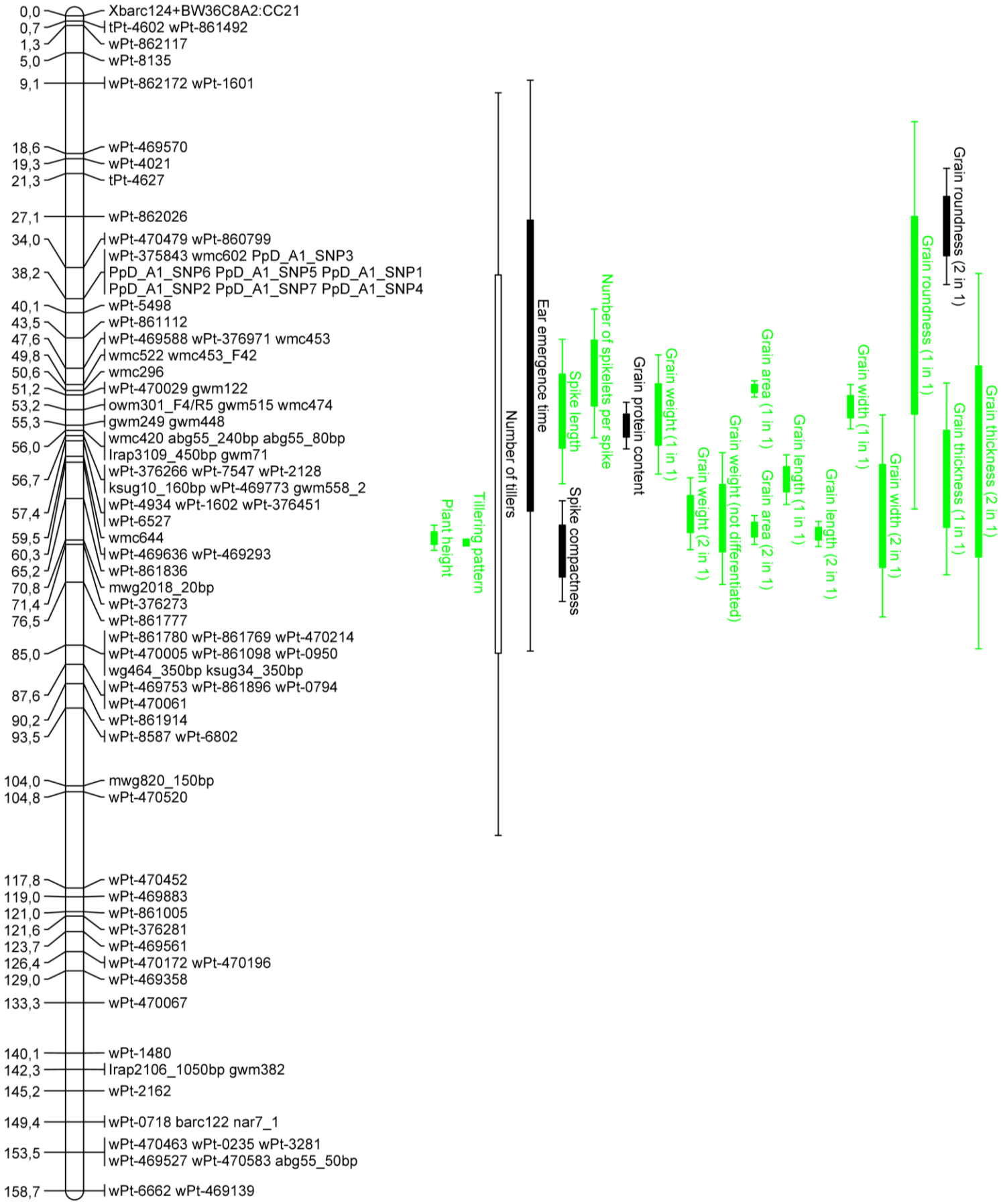
Minor highly significant QTLs are shown as black filled bars, major highly significant QTLs as green filled bars, minor significant QTLs as black open bars, and major significant QTLs as green open bars.

Lines represent the spans of 95% confidence intervals (detailed information available in Appendix 2).

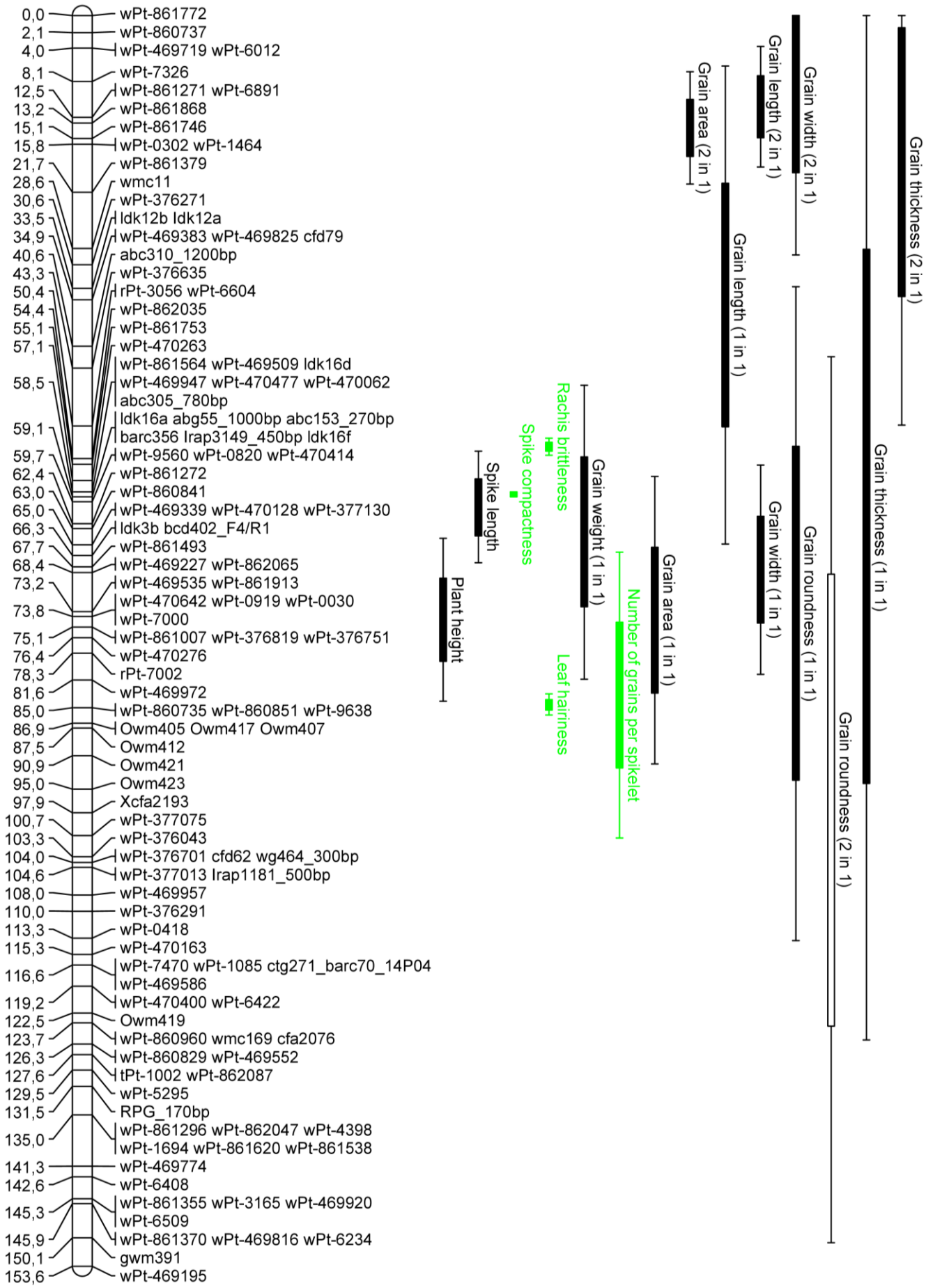
Chromosome 1A^{m}



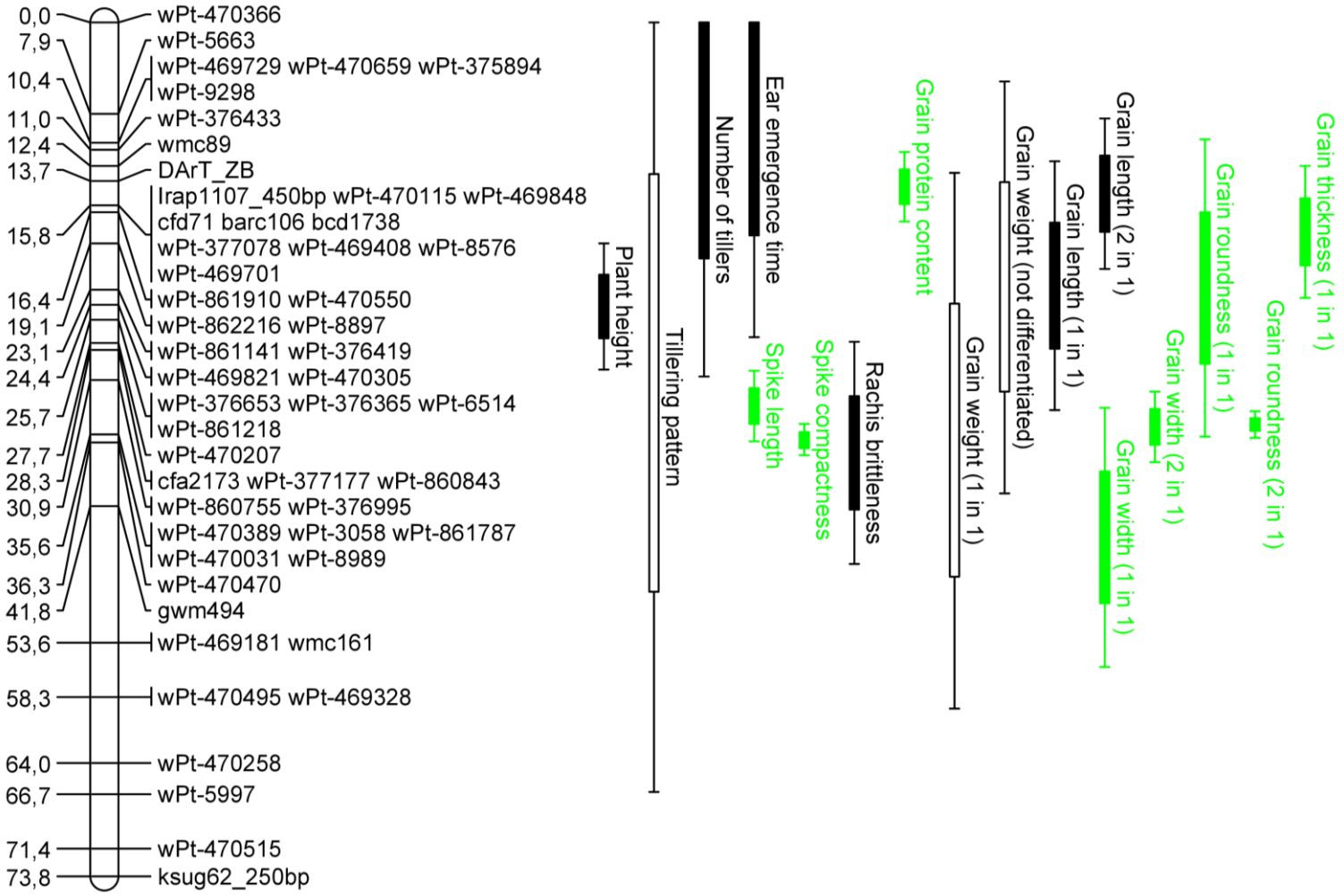
Chromosome 2A^{m}



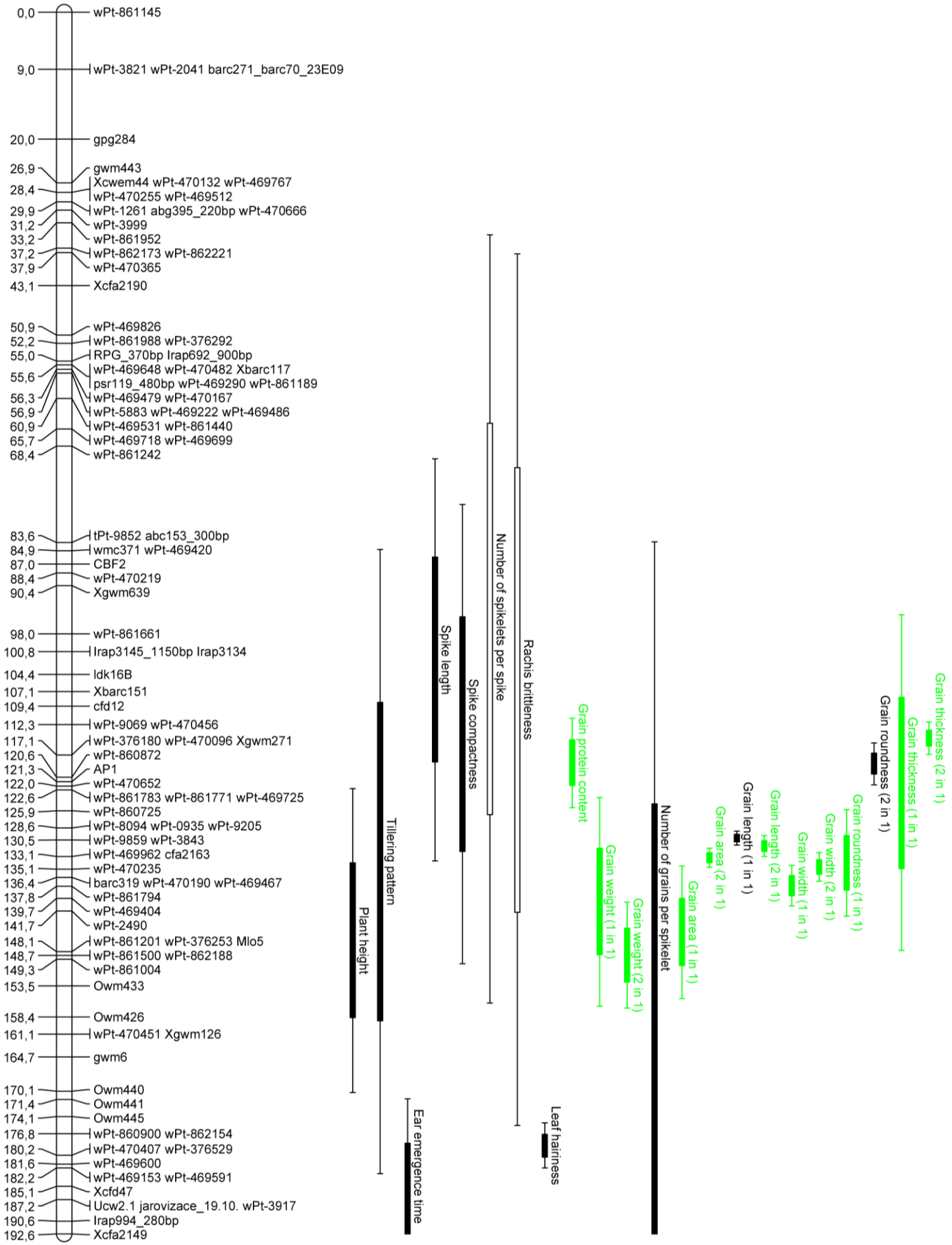
Chromosome 3A^{m}



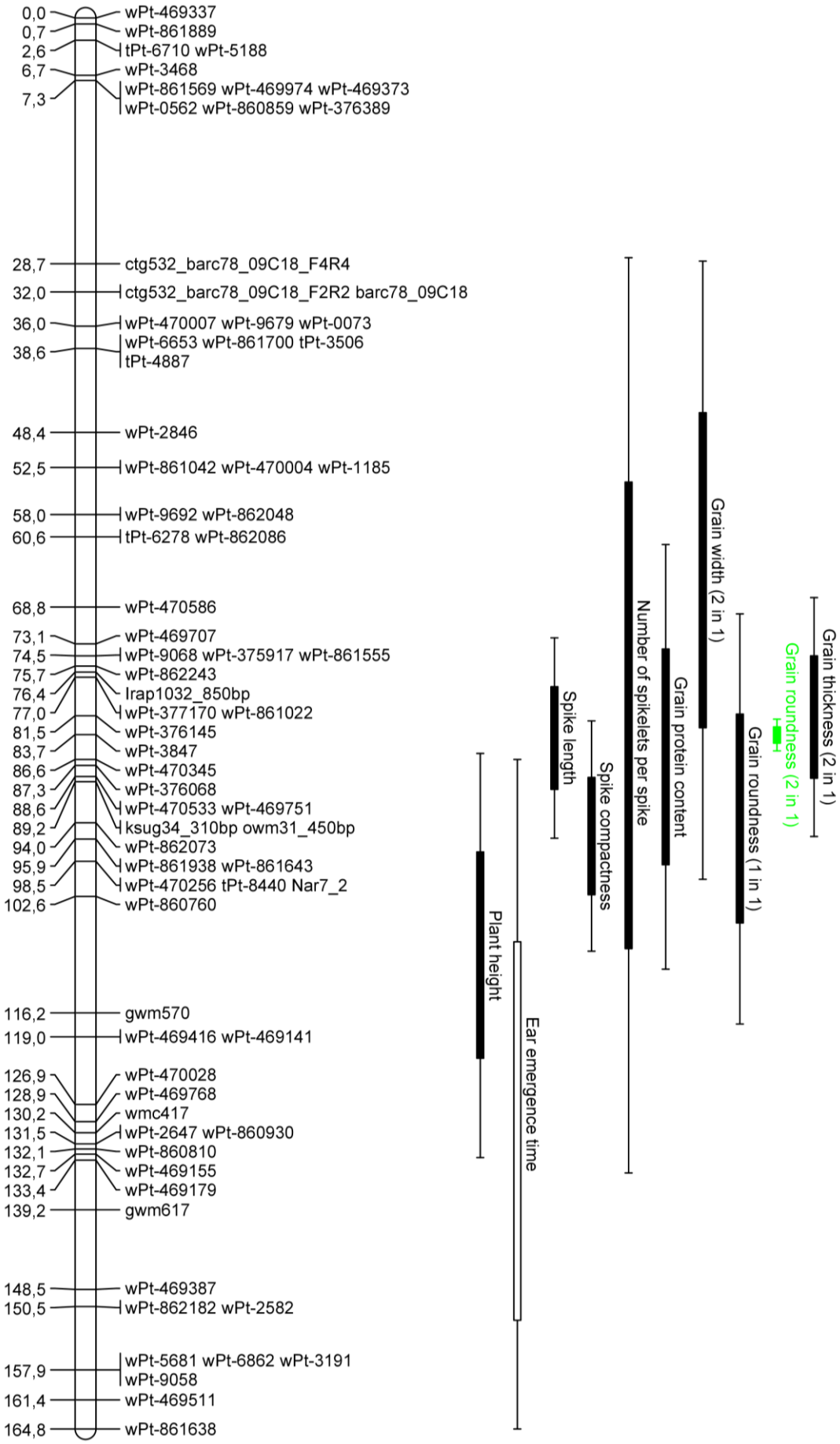
Chromosome 4A^{m}



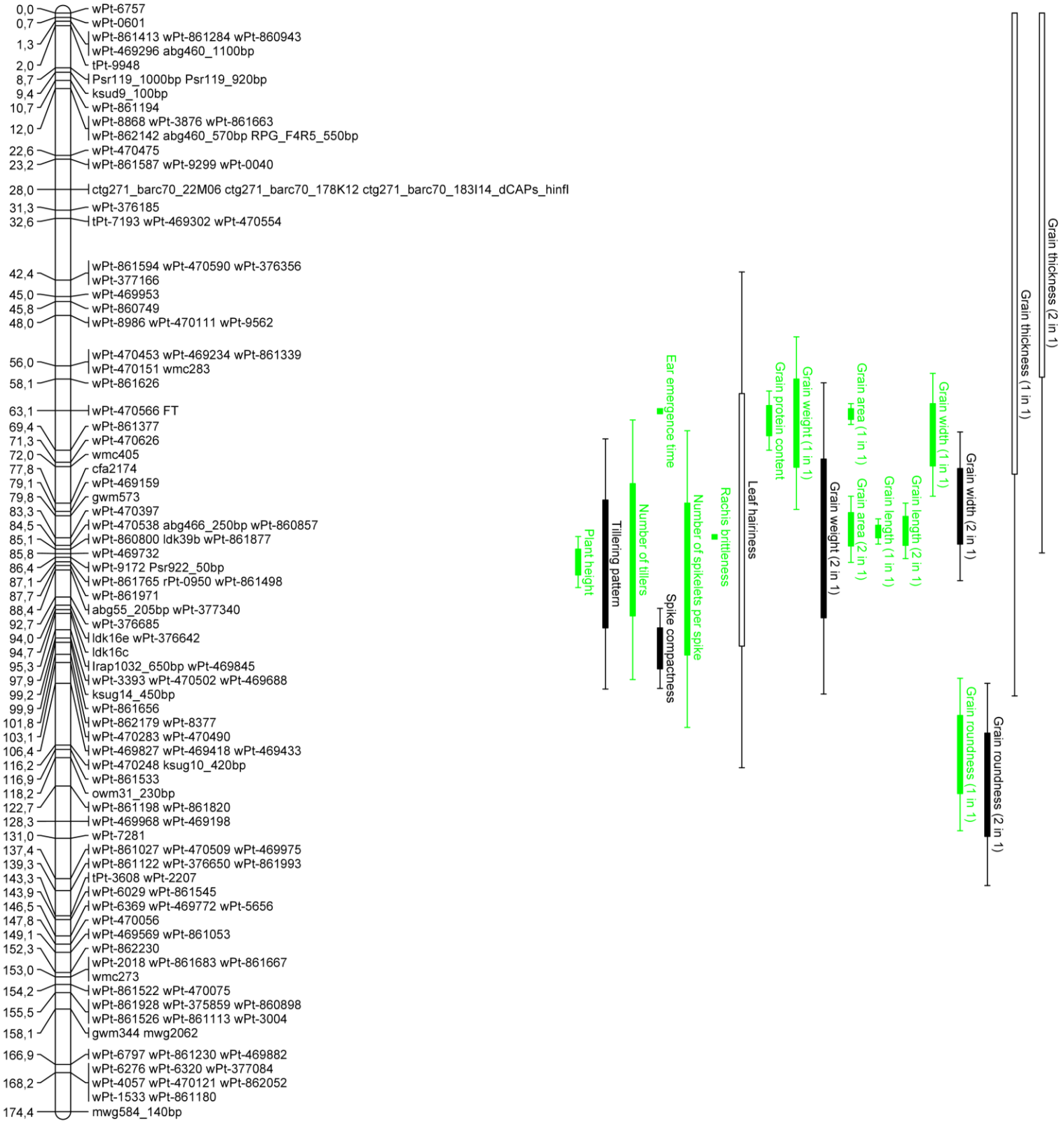
Chromosome 5A^{m}



Chromosome 6A^{m}



Chromosome 7A^{m}



Appendix 2. QTLs detected by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116.

[1] Chromosome on which the QTL was detected (visualized in Appendix 1).

[2] Position of LOD peak with standard deviation (SD).

[3] 95% confidence interval of the QTL span calculated using a bootstrap method.

[4] LOD peak score.

[5] 99% (significant QTLs) and 99.9% (highly significant QTLs) LOD thresholds calculated using a global permutation test.

[6] QTL effects are provided as the percentage of explained variance (PEV) for each of the eight environments 1–8 (E1–8) with SD.

E1–8 represent Stupice (2009), Spring (2009), Spring (2010), Fall (2010), Spring (2011), Fall (2011), Spring (2012), and Italy (2012), respectively. Environments in which the trait has not been assessed are marked as –.

* Chromosomes with QTLs that are considered to be major (PEV > 10% in at least half of the environments).

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Plant height	$1A^m$	113.50±6.87	[100.0;115.2]	11.75	6.29	7.19	3.7±3.4	2.4±2.4	2.8±1.8	1.1±1.6	17.6±5.4	3.0±2.3	9.5±4.6	1.4±1.8
	$2A^m$*	70.53±0.87	[68.8;72.2]	48.25	6.72	16.11	18.0±6.4	30.0±6.8	33.3±4.6	24.3±6.5	10.5±4.4	12.5±4.4	16.8±5.6	22.5±5.9
	$3A^m$	74.22±5.10	[64.2;84.2]	14.59	6.57	7.35	6.9±4.5	5.7±3.9	8.3±3.0	2.9±2.6	5.4±3.3	2.1±2.0	5.9±4.2	11.1±5.2
	$4A^m$	24.54±2.76	[19.1;30.0]	12.47	6.07	7.84	4.5±3.5	5.3±3.6	7.6±2.9	6.3±3.8	4.1±2.9	3.9±2.7	2.0±2.3	1.7±1.9
	$5A^m$	146.31±12.21	[122.4;170.3]	16.53	6.9	8.62	2.6±2.8	2.9±2.6	5.8±2.7	7.8±4.0	6.8±3.8	12.2±4.8	0.9±1.3	9.2±4.4
	$6A^m$	109.47±12.04	[85.9;133.1]	9.76	6.52	8.44	3.2±3.4	5.0±3.6	7.6±3.4	1.5±1.8	3.7±3.4	4.8±3.4	2.9±3.0	2.7±2.6
	$7A^m$*	87.14±2.08	[83.1;91.2]	22.16	6.9	8.66	2.3±2.4	2.7±2.4	10.1±3.3	6.9±4.3	5.5±3.5	28.2±5.7	10.0±5.3	12.7±5.5
Number of tillers	$1A^m$*	109.92±16.68	[77.2;115.2]	8.69	4.96	5.51	–	–	1.5±2.3	1.6±2.3	14.7±6.0	16.7±5.8	9.9±5.5	–
	$2A^m$	60.53±25.56	[10.4;110.7]	5.69	5.33	6.73	–	–	5.0±4.4	2.1±2.6	2.0±2.9	7.7±5.5	14.4±8.0	–
	$4A^m$	9.70±10.68	[0.00;30.64]	6.04	4.85	5.99	–	–	15.2±9.3	3.8±4.0	6.1±4.7	6.2±4.3	5.3±4.5	–
	$7A^m$*	85.19±10.51	[64.6;105.8]	9.35	5.21	6.84	–	–	2.6±3.4	20.4±7.4	8.7±5.7	18.5±7.0	2.1±2.9	–

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Tillering pattern	1A^{m}	111.40±8.20	[95.3;115.2]	16.34	5.64	7.03	–	–	7.4±4.2	4.2±4.3	24.1±8.0	8.3±4.7	14.0±6.5	2.3±3.5
	2A^{m}*	71.15±0.48	SD < 0.5 cM	28.9	5.51	8.36	–	–	22.9±5.9	22.6±7.3	12.4±4.8	20.0±6.2	16.8±5.7	33.0±7.6
	4A^{m}	31.13±18.04	[0.0;66.5]	6.18	5.45	6.51	–	–	10.5±5.7	5.2±5.5	8.7±4.8	2.6±3.0	2.7±3.0	2.2±2.7
	5A^{m}	133.92±25.10	[84.7;183.1]	7.53	5.96	7.45	–	–	8.2±4.9	2.2±3.1	2.9±2.8	11.3±6.5	7.0±4.8	2.3±3.2
	7A^{m}	87.44±10.12	[67.6;107.3]	11.42	5.87	8.49	–	–	2.7±2.4	5.3±4.6	1.1±1.4	17.0±7.0	8.0±4.8	9.6±5.6
Leaf pubescence	3A^{m}*	84.61±0.64	[83.3;85.9]	159.53	7.26	70.54	22.1±5.2	27.0±5.2	78.8±2.4	76.6±4.2	79.4±4.1	74.3±4.4	73.7±3.7	27.9±5.8
	5A^{m}	178.68±1.81	[175.1;182.2]	32.5	6.76	14.11	36.9±5.9	32.9±5.5	0.2±0.3	0.3±0.4	0.2±0.3	6.3±2.4	1.6±1.3	22.8±6.3
	7A^{m}	80.46±20.08	[41.1;119.8]	8.83	6.84	47.49	3.4±2.8	3.7±2.7	2.9±1.7	1.8±1.5	0.7±0.7	1.2±1.2	2.4±1.9	2.4±2.3
Ear emergence time	1A^{m}*	115.15±0.16	SD < 0.5 cM	58.93	5.01	11.64	–	–	33.1±6.2	20.9±6.0	56.3±3.0	13.4±4.6	38.9±5.8	–
	2A^{m}	47.26±19.65	[8.7;85.8]	7.74	5.13	6.5	–	–	2.7±2.4	4.5±3.2	5.5±2.2	2.3±2.4	1.4±1.6	–
	4A^{m}	9.18±9.17	[0.00;27.17]	6.73	4.89	5.94	–	–	1.1±1.5	7.8±3.4	1.3±1.0	5.5±3.6	3.1±2.3	–
	5A^{m}	185.59±7.30	[171.3;192.6]	12.01	4.99	6.07	–	–	2.0±2.1	1.6±1.6	8.7±2.5	1.0±1.3	7.7±3.3	–
	6A^{m}	130.01±22.13	[86.6;164.8]	6.57	5.41	7.36	–	–	2.8±3.0	2.9±2.7	3.5±1.9	5.2±3.6	1.5±1.6	–
	7A^{m}*	63.19±0.40	SD < 0.5 cM	29.47	5.1	6.57	–	–	5.3±3.5	17.0±4.1	8.7±2.3	37.6±5.6	12.1±4.5	–
Spike length	1A^{m}	115.21±0.16	SD < 0.5 cM	14.68	5.17	6.88	–	4.8±2.9	4.2±2.3	0.8±1.1	8.8±3.5	18.1±5.7	12.0±4.8	–
	2A^{m}*	53.41±4.97	[43.7;63.2]	19.83	5.81	6.89	–	13.2±4.7	12.9±3.8	7.3±3.8	12.7±4.5	13.5±5.6	2.5±2.5	–
	3A^{m}	60.39±3.49	[53.5;67.2]	15.28	5.6	6.4	–	8.3±3.7	19.0±4.4	2.1±2.3	2.5±2.0	3.3±2.8	6.8±4.0	–
	4A^{m}*	33.16±1.54	[30.1;36.2]	32.24	5.53	8.3	–	26.1±6.6	30.1±4.6	14.9±4.6	18.6±4.9	9.6±4.7	12.9±5.2	–
	5A^{m}	102.09±16.16	[70.4;133.8]	10.25	5.57	6.41	–	3.6±3.6	4.6±3.0	3.0±3.3	9.1±3.8	7.9±5.2	6.0±5.0	–
	6A^{m}	84.11±5.97	[72.4;95.8]	14.03	5.93	8.33	–	6.8±4.1	2.8±2.0	8.9±4.2	13.0±4.5	1.6±2.1	11.5±5.0	–

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Number of spikelets per spike	1A^{m}*	115.21±0.16	SD < 0.5 cM	34.38	5.94	8.08	11.4±5.5	17.0±5.5	20.2±5.4	7.6±4.1	34.3±5.3	21.4±5.9	16.7±5.2	–
	2A^{m}*	48.29±4.45	[39.6;57.0]	20.52	6.11	7.51	2.6±3.7	15.8±6.1	17.5±5.7	10.1±4.8	9.2±4.1	16.4±6.0	11.3±5.5	–
	5A^{m}	95.63±30.88	[35.1;156.2]	7.22	6.34	7.85	4.8±4.4	1.2±1.7	3.5±3.2	8.9±5.2	4.6±3.5	3.4±3.6	3.4±3.2	–
	6A^{m}	81.46±27.26	[28.0;134.9]	7.58	6	6.93	5.2±5.7	3.1±2.8	2.8±2.7	5.8±4.3	6.4±4.3	1.1±1.9	8.0±5.0	–
	7A^{m}*	89.84±12.01	[66.3;113.4]	13.12	6.39	7.7	12.9±7.0	11.2±5.7	3.0±3.3	10.8±5.7	3.5±2.9	1.2±1.7	11.0±5.1	–
Spike compactness	1A^{m}	70.57±2.01	[66.6;74.5]	23.46	5.82	6.48	–	9.6±4.4	9.7±3.0	2.9±2.2	26.7±5.6	7.4±3.6	8.1±4.4	–
	2A^{m}	72.30±3.49	[65.5;79.1]	12.9	5.77	7.01	–	2.9±2.4	8.2±2.7	4.6±2.5	4.0±2.8	5.5±3.2	3.0±2.7	–
	3A^{m}*	58.80±0.33	SD < 0.5 cM	29	5.69	7.19	–	17.3±5.1	16.8±3.5	11.6±3.8	7.7±3.4	8.0±3.5	4.9±3.4	–
	4A^{m}*	36.06±0.70	[34.7;37.4]	49.51	5.21	6.57	–	17.1±5.3	29.6±3.9	31.4±5.3	15.1±4.9	25.7±5.3	17.6±6.3	–
	5A^{m}	113.81±18.48	[77.6;150.0]	12.73	5.73	7.36	–	5.0±3.6	4.5±2.2	2.6±2.0	3.4±2.6	10.4±4.8	7.0±4.3	–
	6A^{m}	95.52±6.85	[82.1;109.0]	12.1	5.86	6.57	–	3.5±2.8	3.5±1.9	8.5±3.5	3.2±2.6	4.0±2.5	7.4±4.7	–
	7A^{m}	100.84±3.23	[94.5;107.2]	18.28	5.86	7.25	–	7.1±3.8	7.8±2.6	6.9±3.2	7.0±3.3	9.5±3.8	1.8±2.1	–
Rachis brittleness	1A^{m}	98.04±1.64	[94.8;101.2]	19.03	5.65	7.33	–	13.1±4.0	5.4±2.8	2.8±2.2	4.7±2.9	16.4±6.1	8.7±3.9	–
	3A^{m}*	52.97±0.55	[51.9;54.0]	52.31	5.81	9.12	–	30.7±5.7	29.7±5.7	31.3±6.0	41.1±6.4	17.0±5.7	21.8±5.7	–
	4A^{m}	37.20±4.89	[27.6;46.8]	17.24	5.58	7.69	–	10.3±4.3	9.6±4.5	9.3±4.0	7.2±3.6	6.0±3.9	13.8±6.3	–
	5A^{m}	106.83±35.03	[38.1;175.5]	7.2	6.28	8.36	–	1.5±1.4	0.8±1.2	8.3±4.8	3.2±2.6	1.5±2.0	5.3±3.8	–
	7A^{m}*	83.17±0.35	SD < 0.5 cM	27.52	5.76	7.21	–	13.8±3.7	19.8±4.9	11.2±4.5	5.2±3.1	17.6±6.6	10.4±4.5	–
Number of grains per spikelet	1A^{m}*	51.80±2.76	[46.4;57.2]	17.97	5.57	7.83	–	–	16.9±6.3	15.1±6.2	7.4±4.2	6.3±4.5	11.4±5.1	18.3±5.4
	3A^{m}*	83.49±8.95	[65.9;101.0]	17.34	5.62	7.58	–	–	10.3±4.9	11.6±6.8	11.2±5.8	12.3±6.2	9.4±5.3	19.6±5.8
	5A^{m}	167.75±42.96	[83.5;192.6]	8.89	5.86	8.31	–	–	5.2±3.9	3.1±3.4	5.3±3.9	2.6±3.0	12.6±5.7	8.2±4.3

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Grain weight (1 in 1)	1A^{m}	103.35±25.16	[54.0;115.2]	7.08	5.86	6.75	–	–	5.1±3.4	1.4±2.5	12.5±5.7	1.8±2.4	3.3±2.8	1.7±2.1
	2A^{m}*	53.85±4.11	[45.8;61.9]	26.66	5.45	6.73	–	–	19.1±6.1	20.3±6.1	4.7±3.1	15.5±5.6	16.5±5.0	17.8±5.5
	3A^{m}	63.41±9.20	[45.4;81.5]	11.76	5.82	6.96	–	–	2.3±2.4	6.6±4.1	12.2±4.7	9.9±5.3	6.2±3.8	4.8±3.3
	4A^{m}	36.12±11.80	[13.0;59.3]	5.46	5.34	7.73	–	–	1.3±2.1	1.2±1.7	3.6±3.5	9.7±7.0	2.2±2.3	8.2±5.8
	5A^{m}*	140.22±8.39	[123.8;156.7]	15.16	6.1	7.2	–	–	9.7±4.9	6.4±4.3	12.1±5.8	10.4±6.0	10.6±5.2	10.6±5.0
	7A^{m}*	65.12±7.00	[51.4;78.8]	20.97	6.04	7.69	–	–	19.4±5.4	17.4±5.5	10.9±5.1	4.9±3.6	12.7±5.1	10.0±4.3
Grain weight (2 in 1)	2A^{m}*	67.29±2.47	[62.4;72.1]	27.58	6.2	9.03	–	–	11.8±3.8	26.7±6.5	4.3±3.6	22.3±6.0	25.9±6.0	20.2±6.1
	5A^{m}*	148.66±4.25	[140.3;157.0]	20.61	5.69	6.98	–	–	6.2±3.2	13.8±4.8	14.8±5.8	14.3±5.3	11.7±5.2	12.9±5.0
	7A^{m}	83.36±12.60	[58.7;108.1]	11.69	5.75	7.17	–	–	7.0±3.2	15.9±5.4	1.8±2.4	11.0±5.8	3.4±3.0	3.0±2.8
Grain weight (not differentiated)	1A^{m}*	76.35±23.89	[29.5;115.2]	4.48	3.58	4.72	10.4±5.8	10.0±5.5	–	–	–	–	–	–
	2A^{m}*	67.88±4.53	[59.0;76.8]	10.6	4.1	5.08	18.6±6.4	32.2±6.6	–	–	–	–	–	–
	4A^{m}	22.89±9.06	[5.1;40.7]	3.49	3.24	3.94	9.5±6.3	7.9±4.8	–	–	–	–	–	–

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Grain area (1 in 1)	1A^{m}	51.86±9.14	[33.9;69.8]	16.93	5.96	8.25	–	–	3.5±3.1	8.0±4.5	11.7±4.5	11.2±5.7	9.7±3.7	5.5±3.3
	2A^{m}*	50.37±0.55	[49.3;51.5]	37.36	6.36	7.7	–	–	20.6±6.1	12.6±4.0	10.1±4.7	23.9±5.5	18.0±4.4	18.5±5.5
	3A^{m}	74.24±8.99	[56.6;91.9]	11.76	5.89	9.94	–	–	4.5±3.2	2.0±1.7	6.0±3.6	7.7±3.9	7.4±3.1	6.5±3.4
	5A^{m}*	145.02±5.32	[134.6;155.5]	25.13	5.97	7.24	–	–	7.1±4.9	9.1±5.0	10.9±4.4	7.8±4.1	14.2±4.0	12.6±4.6
	7A^{m}*	63.64±0.83	[62.0;65.3]	37.04	5.94	9.51	–	–	22.0±6.3	25.7±4.8	16.6±5.5	9.8±3.8	18.3±4.2	18.3±5.2
Grain area (2 in 1)	1A^{m}	60.41±3.92	[52.7;68.1]	18.54	6.08	7.27	–	–	3.6±2.4	6.4±3.2	7.0±3.9	13.2±4.2	8.3±3.2	5.3±2.9
	2A^{m}*	69.43±1.00	[67.5;71.4]	41.15	5.81	7.9	–	–	22.1±5.2	15.9±4.5	5.7±3.5	18.8±4.5	24.8±4.3	24.8±5.1
	3A^{m}	13.81±3.51	[6.9;20.7]	19.67	5.77	6.92	–	–	7.7±3.4	3.8±2.6	9.1±3.9	9.5±3.8	7.8±3.4	7.8±3.4
	5A^{m}*	133.29±0.78	[131.8;134.8]	39.67	5.67	7.87	–	–	12.4±3.9	10.2±4.4	22.4±5.4	25.8±4.7	20.7±4.3	15.6±4.7
	7A^{m}*	81.95±2.66	[76.7;87.2]	30.75	5.84	6.95	–	–	16.1±5.0	23.3±5.4	8.9±4.4	9.7±4.2	9.3±3.3	10.7±3.8
Grain length (1 in 1)	1A^{m}	89.46±11.88	[66.2;112.8]	13.63	5.59	7.27	–	–	12.1±4.9	3.2±2.1	5.7±4.4	8.7±3.6	3.7±2.7	7.2±4.3
	2A^{m}*	62.63±1.72	[59.3;66.0]	36.93	5.47	8.26	–	–	20.8±5.2	11.5±3.7	9.5±5.4	16.5±4.3	19.5±4.7	25.5±5.1
	3A^{m}	35.57±14.97	[6.2;64.9]	11.78	6.3	8.03	–	–	1.7±1.8	3.4±2.3	5.0±3.8	9.7±3.7	7.9±3.7	4.2±3.1
	4A^{m}	22.77±5.48	[12.0;33.5]	7.3	5.29	6.95	–	–	1.4±1.6	8.8±3.4	1.6±1.9	3.5±2.5	3.2±2.2	1.3±1.7
	5A^{m}	130.20±0.58	[129.1;131.3]	19.89	5.92	6.7	–	–	7.6±3.1	7.0±2.9	4.9±3.7	16.8±3.9	14.6±4.4	5.9±3.4
	7A^{m}*	82.29±1.01	[80.3;84.3]	40.92	5.49	8.67	–	–	24.7±5.3	37.6±4.5	11.2±5.6	19.1±4.2	20.5±4.4	12.6±4.6
Grain length (2 in 1)	1A^{m}	61.38±0.80	[59.8;62.9]	18.11	5.5	7.55	–	–	2.4±1.8	6.1±2.7	6.7±3.3	15.4±4.6	6.9±2.9	4.9±2.7
	2A^{m}*	69.99±0.85	[68.3;71.7]	38.84	5.53	9.77	–	–	23.9±4.9	10.1±3.9	5.3±3.1	19.7±4.6	22.1±4.5	22.5±5.4
	3A^{m}	11.21±3.77	[3.8;18.6]	17.31	5.75	6.64	–	–	8.9±3.8	2.9±2.2	8.2±3.7	7.5±3.5	7.4±3.3	7.5±3.5
	4A^{m}	14.81±3.30	[8.3;21.3]	19.14	5.42	8.3	–	–	5.2±2.8	13.9±3.9	5.2±3.1	8.5±3.5	4.8±2.4	6.7±3.4
	5A^{m}*	131.44±0.85	[129.8;133.1]	25.4	5.87	6.99	–	–	5.8±2.7	5.2±3.0	16.9±4.7	19.8±4.6	13.9±4.1	6.9±3.3
	7A^{m}*	82.20±2.26	[77.8;86.6]	36.27	6.06	10.89	–	–	16.2±4.8	28.8±4.3	15.4±5.2	9.9±4.3	14.7±4.4	9.6±3.9

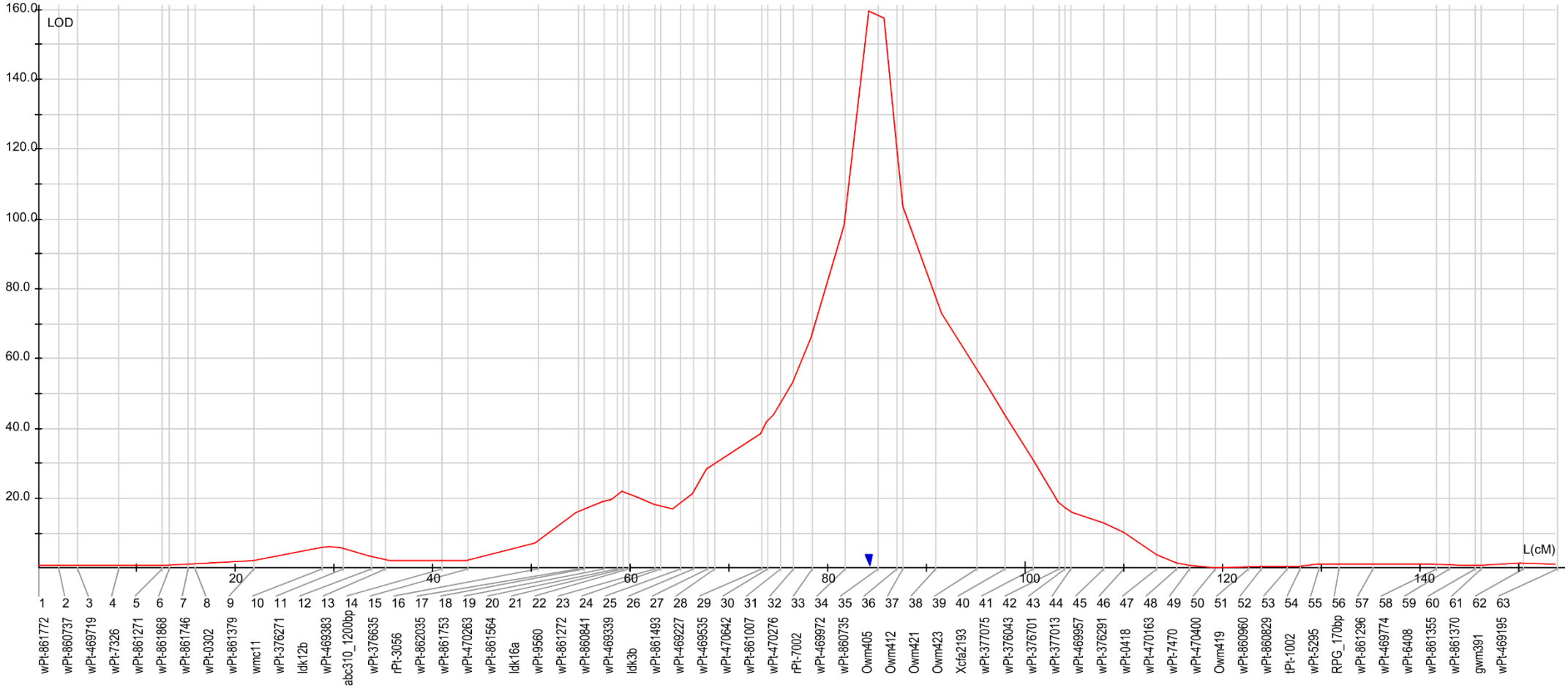
Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Grain width (1 in 1)	1A^{m}	111.59±9.28	[93.4;115.2]	16.02	5.52	8.14	–	–	9.0±3.9	2.5±2.2	11.9±4.2	5.2±3.2	6.7±3.0	8.3±3.4
	2A^{m}*	52.80±1.52	[49.8;55.8]	35.21	5.86	7.23	–	–	20.7±5.5	17.0±6.1	10.8±4.5	23.3±6.1	19.0±5.3	16.0±4.0
	3A^{m}	68.01±6.56	[55.2;80.9]	16.55	5.69	7.58	–	–	4.8±2.8	1.9±1.9	8.1±3.6	8.9±4.2	10.1±3.8	10.7±3.5
	4A^{m}*	44.50±5.73	[33.3;55.7]	12.68	5.16	7.43	–	–	5.4±3.6	11.4±6.2	11.0±5.0	8.7±5.2	3.7±3.1	13.8±5.8
	5A^{m}*	137.68±1.62	[134.5;140.9]	23.16	5.9	8.08	–	–	9.3±3.8	5.1±3.2	9.4±3.9	12.5±4.9	14.5±4.2	12.3±3.8
	7A^{m}*	66.94±4.99	[57.2;76.7]	22.44	5.85	7.91	–	–	12.5±4.7	16.7±5.3	10.0±4.3	3.9±3.2	12.0±4.6	6.4±3.6
Grain width (2 in 1)	1A^{m}*	47.10±8.98	[29.5;64.7]	17.02	5.56	7.02	–	–	2.8±2.4	6.9±3.8	10.0±5.7	17.0±5.9	11.6±4.0	5.6±2.8
	2A^{m}*	67.53±6.97	[53.9;81.2]	24.65	5.75	6.58	–	–	13.2±4.7	13.3±4.7	2.0±2.1	6.6±3.9	12.6±4.5	12.1±3.9
	3A^{m}	8.86±10.49	[0.00;29.43]	8.81	5.83	6.69	–	–	2.9±2.7	3.7±2.7	5.7±3.2	6.1±3.9	4.7±2.9	3.5±2.1
	4A^{m}*	34.93±1.55	[31.9;38.0]	24.46	5.59	7.46	–	–	14.7±5.5	9.5±4.1	14.8±5.3	5.0±3.3	9.8±4.3	19.5±5.1
	5A^{m}*	134.74±1.15	[132.5;137.0]	29.14	6.48	8.5	–	–	12.5±4.4	8.0±3.7	14.7±4.5	22.4±5.7	13.9±4.7	13.8±3.9
	6A^{m}	64.51±18.39	[28.4;100.6]	7.1	5.73	7.03	–	–	2.1±2.0	2.7±2.2	2.7±2.4	2.3±2.5	4.0±2.7	6.4±3.2
	7A^{m}	78.32±6.00	[66.5;90.1]	20.28	5.9	8.33	–	–	9.1±4.0	17.9±4.9	4.8±3.1	13.5±7.2	6.6±3.3	7.6±3.1
Grain roundness (1 in 1)	1A^{m}	53.64±16.61	[21.1;86.2]	7.9	5.57	6.94	–	–	2.6±2.5	2.2±2.4	2.7±2.5	4.9±3.6	4.9±3.1	3.5±2.7
	2A^{m}*	40.47±13.34	[14.3;66.6]	18.54	6.24	7.41	–	–	6.7±3.8	13.8±5.4	8.7±3.8	13.3±5.0	12.4±4.4	4.2±2.9
	3A^{m}	73.40±20.48	[33.3;113.6]	6.81	5.72	6.62	–	–	4.2±3.2	1.0±1.3	3.5±2.6	4.1±3.2	4.8±3.0	7.7±4.1
	4A^{m}*	22.93±6.54	[10.1;35.8]	27.82	5.41	6.11	–	–	20.5±5.3	17.8±5.2	14.8±4.6	9.1±4.3	8.6±3.7	17.7±5.4
	5A^{m}*	134.07±4.29	[125.7;142.5]	14.58	5.76	7.57	–	–	12.4±4.7	7.9±3.8	8.4±3.8	15.9±5.2	14.1±4.2	13.8±4.9
	6A^{m}	93.53±12.20	[69.6;117.5]	13.93	5.33	6.99	–	–	3.8±2.9	5.7±3.3	8.6±3.8	8.4±4.4	8.9±3.7	2.4±2.3
	7A^{m}*	117.68±6.18	[105.6;129.8]	19.56	5.54	8.64	–	–	8.1±4.1	10.6±4.4	10.0±4.0	11.0±4.6	6.9±3.3	10.1±4.3
Grain roundness (2 in 1)	1A^{m}	49.24±3.50	[42.4;56.1]	11.62	5.32	6.97	–	–	3.7±2.5	3.6±1.9	3.2±1.9	4.1±3.5	5.8±2.4	1.4±1.3
	2A^{m}	28.41±4.00	[20.6;36.3]	15.89	5.41	7.08	–	–	3.3±2.3	9.0±2.8	3.6±2.0	11.8±5.6	3.2±1.9	4.6±2.5
	3A^{m}	96.31±27.76	[41.9;150.7]	6.66	5.83	7.37	–	–	1.0±1.7	0.7±0.9	4.1±2.4	1.5±2.2	2.3±1.6	2.8±2.1
	4A^{m}*	34.74±0.58	[33.6;35.9]	61	5.41	9.6	–	–	36.3±4.6	38.0±5.0	27.2±6.0	27.5±6.9	23.1±5.5	37.9±4.6
	5A^{m}	118.47±1.68	[115.2;121.8]	24.19	6.14	8.48	–	–	6.5±3.1	4.4±2.3	7.7±2.6	6.2±4.0	12.0±3.2	6.4±2.7
	6A^{m}*	83.73±0.95	[81.9;85.6]	37.98	5.57	6.73	–	–	12.7±3.7	10.0±2.9	10.1±2.9	9.3±4.3	18.5±3.6	10.6±3.3
	7A^{m}	122.47±8.19	[106.4;138.5]	20.53	5.9	8.36	–	–	5.0±2.8	6.0±2.5	7.8±3.0	5.5±3.9	3.2±2.1	11.5±3.4

Trait	Chr.[1]	Position ± SD [cM][2]	Confidence interval (95%)[3]	LOD[4]	LOD thr.[5]		PEV E1 ± SD [6]	PEV E2 ± SD [6]	PEV E3 ± SD [6]	PEV E4 ± SD [6]	PEV E5 ± SD [6]	PEV E6 ± SD [6]	PEV E7 ± SD [6]	PEV E8 ± SD [6]
					99%	99.9%								
Grain thickness (1 in 1)	2A^{m}*	62.55±6.59	[49.6;75.5]	17.25	5.88	7.13	–	–	13.2±6.5	15.3±6.8	2.1±2.3	17.7±6.7	22.5±6.7	7.4±5.0
	3A^{m}	61.51±32.81	[0.0;125.8]	8.24	6.1	7.2	–	–	4.1±4.3	4.7±4.2	11.2±5.3	6.4±4.9	4.8±3.7	4.2±3.5
	4A^{m}*	18.10±2.93	[12.4;23.8]	16.83	5.35	6.45	–	–	12.0±5.2	7.8±4.6	23.1±6.3	2.6±3.1	6.2±3.4	15.4±6.2
	5A^{m}*	121.46±13.50	[95.0;147.9]	12.69	5.81	6.5	–	–	7.8±4.5	1.4±1.8	10.7±4.7	17.1±7.0	12.0±4.9	5.6±4.1
	7A^{m}	36.43±36.72	[0.0;108.4]	5.97	5.68	6.7	–	–	4.0±4.1	9.5±5.5	2.1±2.4	6.3±5.0	5.9±4.0	2.4±2.9
Grain thickness (2 in 1)	1A^{m}	51.03±17.57	[16.6;85.5]	7.87	5.83	6.57	–	–	1.5±2.3	6.7±4.4	1.5±2.2	4.6±3.6	10.9±5.3	6.1±4.7
	2A^{m}*	60.16±12.91	[34.8;85.5]	18.34	6	7.24	–	–	16.7±7.0	15.7±6.9	2.9±2.7	19.7±6.0	13.4±5.4	3.8±3.9
	3A^{m}	18.01±16.48	[0.0;50.3]	9.44	5.9	7.45	–	–	7.3±4.0	6.3±3.5	4.3±3.1	9.1±4.9	3.5±2.6	3.6±3.1
	5A^{m}*	114.46±1.29	[111.9;117.0]	21.41	5.96	7.03	–	–	15.5±5.8	7.1±3.6	14.3±5.3	22.3±9.0	16.3±4.8	13.0±5.4
	6A^{m}	81.66±7.13	[67.7;95.6]	11.67	5.7	6.97	–	–	2.7±2.4	2.6±2.5	13.7±5.2	14.1±6.7	5.2±3.0	8.4±4.5
	7A^{m}	28.75±29.02	[0.0;85.7]	7	5.7	7.65	–	–	3.4±3.2	6.6±4.2	3.8±4.3	4.4±4.2	7.5±4.4	3.1±3.7
Grain protein content	1A^{m}	65.87±30.71	[5.6;115.2]	8.52	4.49	5.85	–	4.1±3.4	11.8±5.0	9.4±4.9	2.4±2.4	–	–	–
	2A^{m}	55.35±1.59	[52.2;58.5]	10.77	4.59	5.65	–	1.3±1.6	1.9±2.4	14.6±4.7	13.6±4.2	–	–	–
	4A^{m}*	14.21±1.53	[11.2;17.2]	15.69	4.21	6.66	–	21.7±5.8	7.1±4.1	15.9±4.4	4.7±2.7	–	–	–
	5A^{m}*	118.32±3.59	[111.3;125.4]	16.66	4.94	5.83	–	16.4±5.4	13.5±5.4	4.5±2.6	19.9±5.7	–	–	–
	6A^{m}	86.29±12.63	[61.5;111.1]	6.59	4.97	5.82	–	7.5±4.1	7.6±4.3	2.2±1.8	5.2±3.0	–	–	–
	7A^{m}*	64.69±2.41	[60.0;69.4]	17.09	4.84	5.84	–	4.5±3.2	8.7±5.1	18.7±4.7	17.5±4.7	–	–	–

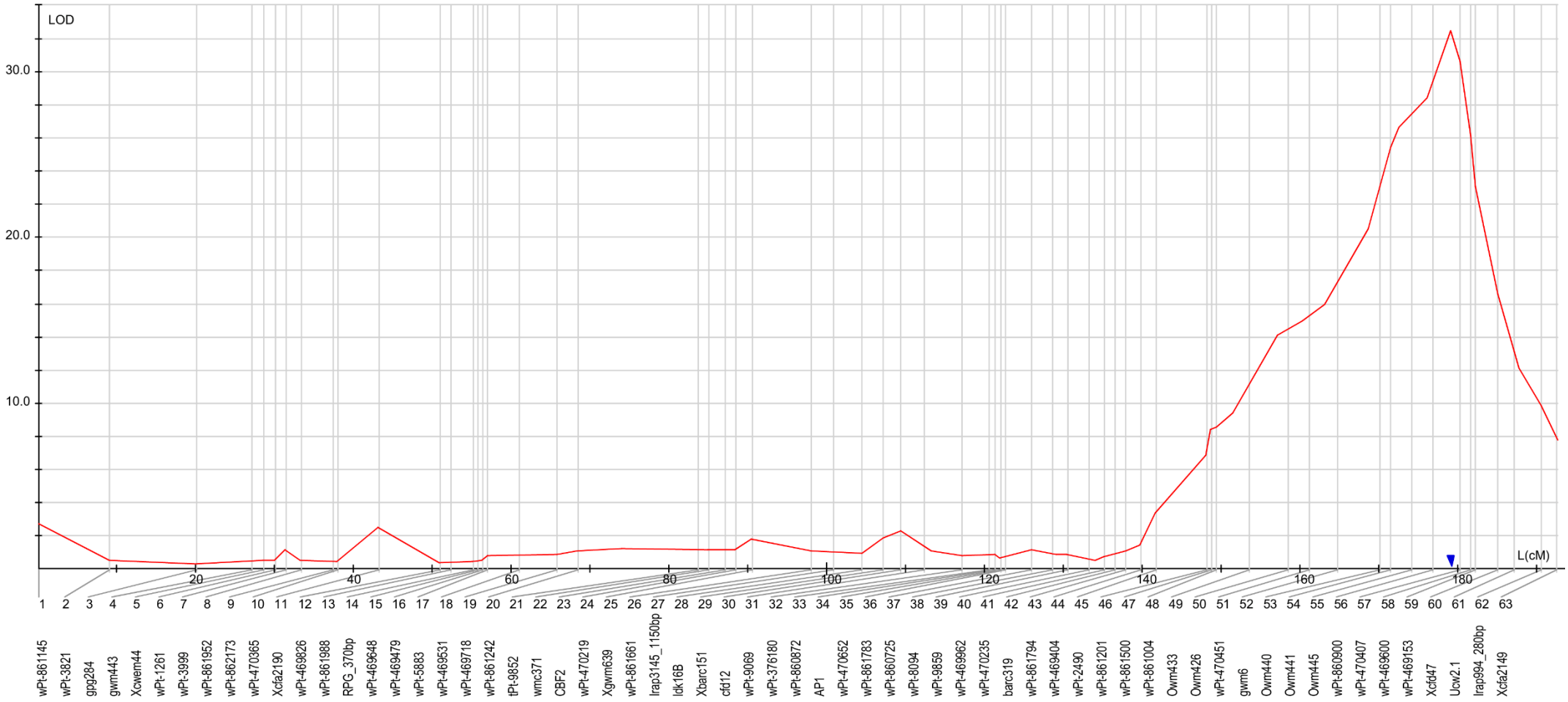
Appendix 3. Highly significant QTLs for leaf pubescence mapped on $3A^m$ and $5A^m$ by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116 using skeleton linkage map.

Skeleton linkage map without attached markers was used for the analysis. Markers and their positions in cM are on the *x* axis, while their LOD scores are on the *y* axis. Blue arrow marks the LOD peak.

Chromosome $3A^m$



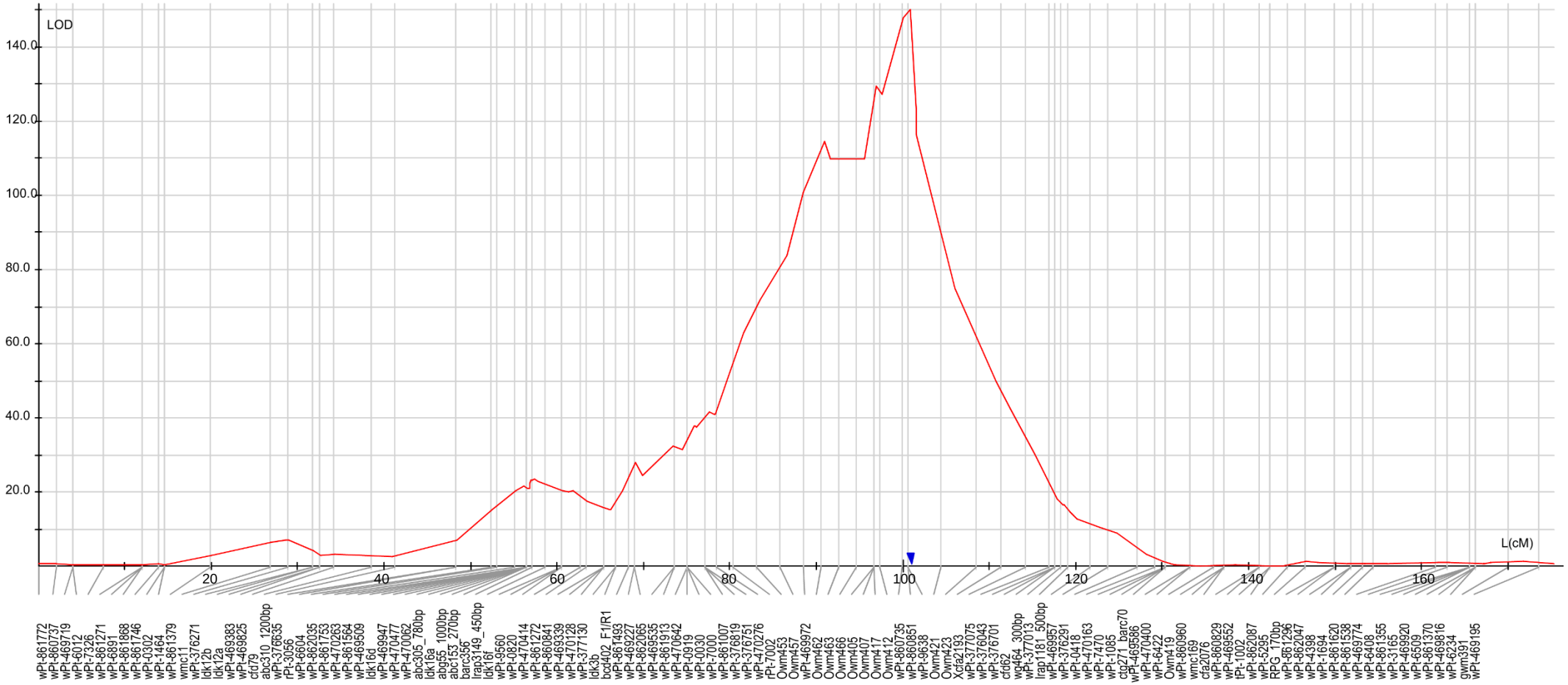
Chromosome 5A^{m}



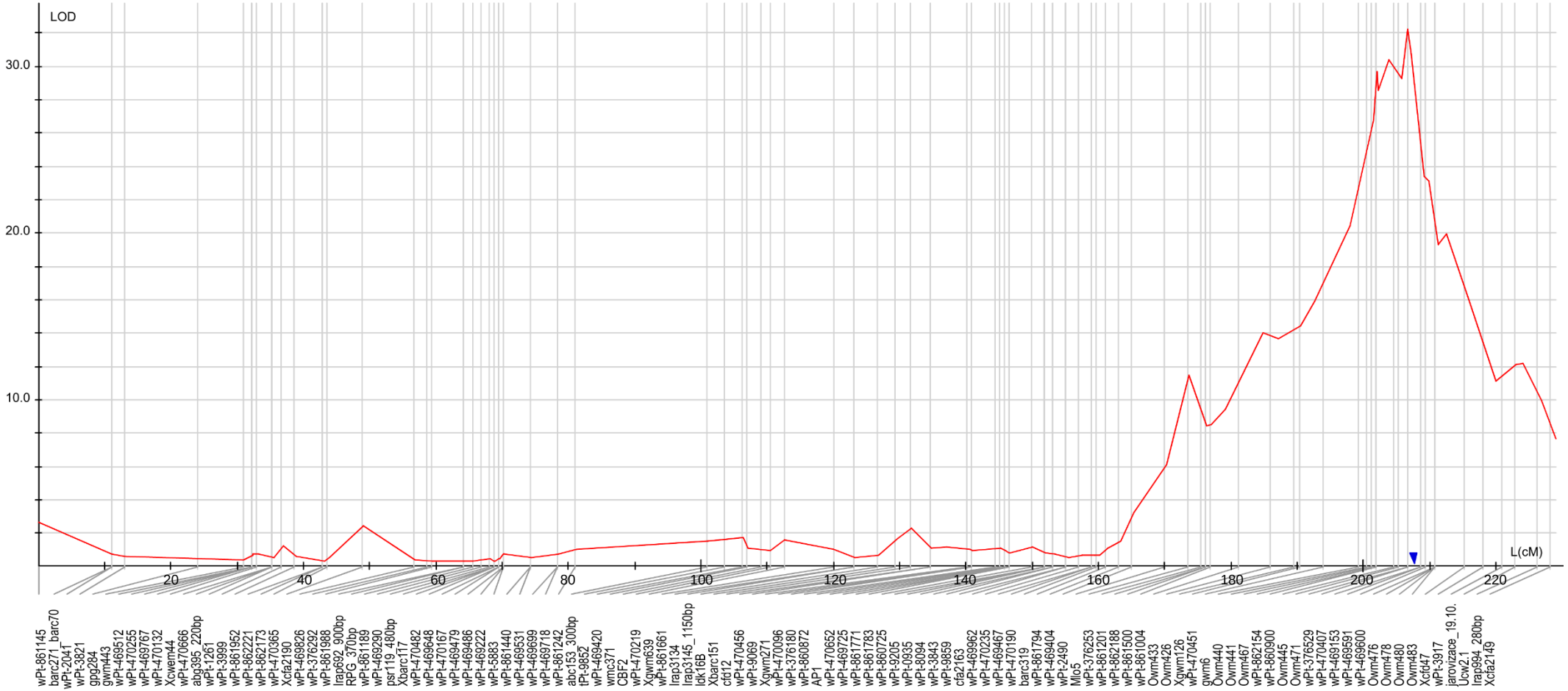
Appendix 4. Highly significant QTLs for leaf pubescence mapped on 3A^{m} and 5A^{m} by multiple environment multiple interval mapping in *Triticum monococcum* F$_{12}$ RIL population derived from DV92×G3116 using adjusted global linkage map.

Adjusted global linkage map with all markers and an additional set of markers (*Owm453*, *Owm457*, *Owm462*, *Owm463*, *Owm466*, *Owm467*, *Owm471*, *Owm476*, *Owm478*, *Owm480*, and *Owm483*) was used for the analysis. Markers and their positions in cM are on the *x* axis, while their LOD scores are on the *y* axis. Blue arrow marks the LOD peak. Note that the genetic distances and marker positions in the graphs and table below are different from that in Appendix 1, 2, and 3 because attached markers including the additional *Owm* markers inflate the map (e.g., markers *Owm462–Owm466* on 3A^{m}) and skew the calculations.

Chromosome 3A^{m}



Chromosome 5A^{m}



Highly significant QTLs for leaf pubescence mapped on $3A^m$ and $5A^m$ by multiple environment multiple interval mapping in *Triticum monococcum* F_{12} RIL population derived from DV92×G3116 using global linkage map

[1] Chromosome on which the QTL was detected.

[2] Position of LOD peak with standard deviation (SD).

[3] 95% confidence interval of the QTL span calculated using a bootstrap method.

[4] LOD peak score.

[5] 99% (significant QTLs) and 99.9% (highly significant QTLs) LOD thresholds calculated using a global permutation test.

[6] QTL effects are provided as the percentage of explained variance (PEV) for each of the eight environments 1–8 (E1–8) with SD.

E1–8 represent Stupice (2009), Spring (2009), Spring (2010), Fall (2010), Spring (2011), Fall (2011), Spring (2012), and Italy (2012), respectively.

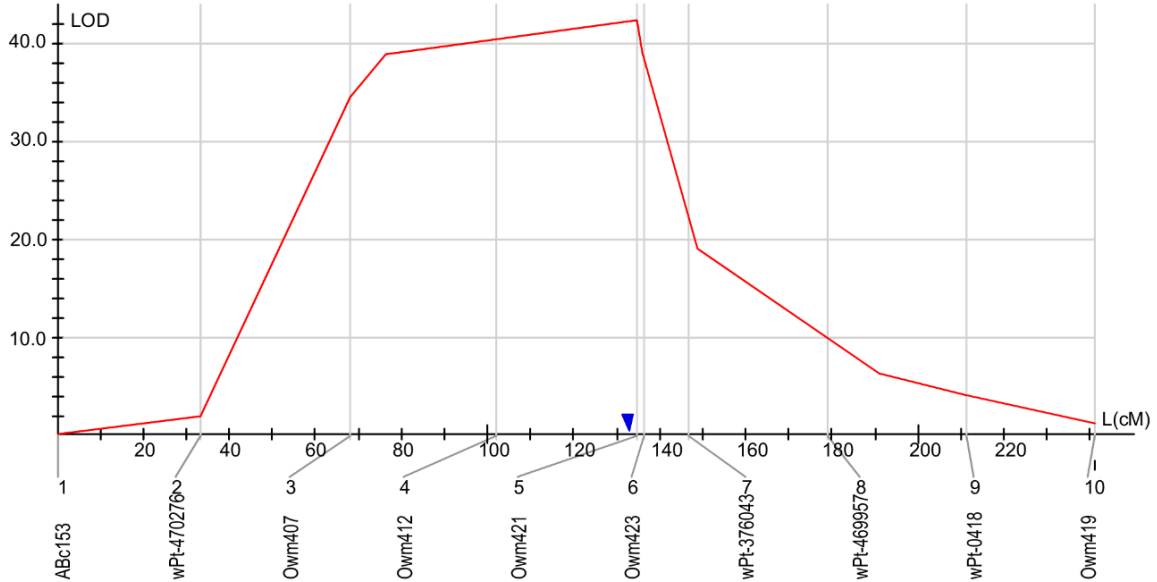
* Chromosomes with QTLs that are considered to be major (PEV > 10% in at least half of the environments).

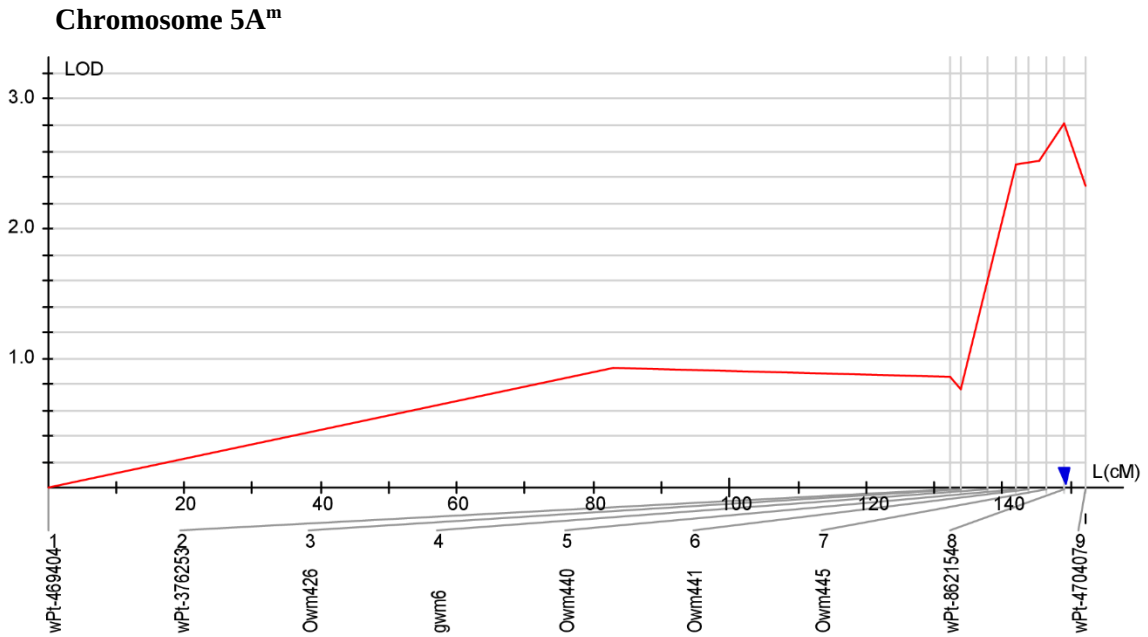
Trait	**Chr.**[1]	**Position ± SD [cM]**[2]	**Confidence interval (95%)**[3]	**LOD**[4]	**LOD thr.**[5]		**PEV E1 ± SD** [6]	**PEV E2 ± SD** [6]	**PEV E3 ± SD** [6]	**PEV E4 ± SD** [6]	**PEV E5 ± SD** [6]	**PEV E6 ± SD** [6]	**PEV E7 ± SD** [6]	**PEV E8 ± SD** [6]
					99%	**99.9%**								
Leaf pubescence	$3A^m$*	100.53±0.46	SD < 0.5 cM	149.95	7.70	23.81	23.5±5.4	28.4±5.3	78.9±2.2	75.4±4.1	78.6±4.2	73.4±4.3	72.5±3.8	28.5±6.1
	$5A^m$	205.51±2.05	[201.5;209.5]	32.24	7.19	10.16	35.2±5.5	31.5±5.7	0.2±0.3	0.5±0.5	0.3±0.4	6.6±3.0	1.9±1.5	22.1±6.2

Appendix 5. Verification of the QTLs for leaf pubescence on 3A^m and 5A^m by single environment multiple interval mapping in *Triticum monococcum* F_2 verification mapping populations.

The QTL for leaf pubescence on 3A^m was verified using all verification mapping populations (144×246, 246×144, DV92×113, and DV92×165), while the QTL on 5A^m was verified only on 144×246 and 246×144 because the other two populations are not polymorphic in the QTL region. Markers and their positions in cM are on the *x* axis, while their LOD scores are on the *y* axis. Blue arrow marks the LOD peak. Note that the marker distances do not reflect the size of chromosomes or QTL positions on them because only a fraction of markers was genotyped on verification mapping populations and was used for the analysis.

Chromosome 3A^m





Verification of QTLs for leaf pubescence on $3A^m$ and $5A^m$

[1] Chromosome on which the QTL was verified.

[2] LOD peak score.

[3] 99% (significant QTLs) and 99.9% (highly significant QTLs) LOD thresholds calculated using a global permutation test.

[4] QTL effects are provided as the percentage of explained variance (PEV) with standard deviation (SD).

Chr.[1]	LOD[2]	LOD thr.[3]		PEV ± SD [4]
		99%	99.9%	
$3A^m$	42.35	4.05	9.83	22.8±3.3
$5A^m$	2.82	2.80	7.83	6.0±4.7

Appendix 6. *Owm400–Owm448* PCR markers.

* LP – length polymorphism; PA – presence-absence polymorphism; SNP – single nucleotide polymorphism (the position of the SNPs is calculated from the beginnings of the corresponding PCR products).

The empty cells in the last two columns mean either no PCR product or no polymorphism.

Marker	Based on SNP markers	Forward (F) and reverse (R) primer sequences (5′→3′)		Amplicon length [bp]	Polymorphism*
Owm400	*RAC875_c13976_509*	F:	GGGATTCAATGGGGTTCAG		
		R:	ATCCTCTTGGTGCAAACTGC		
Owm401		F:	GCAGTTTGCACCAAGAGGAT	~500	
		R:	CTTTTCGGGATGAACATTGG		
Owm402		F:	CAGCCATATCCATTTCATCG	~550	
		R:	TCTTGAGCGAGTTAGGCAAA		
Owm403		F:	CCAATGCTGGATATGCCTCT		
		R:	GTGCGCTTGAAGTGTGTCTC		
Owm404	*wsnp_BQ167580A_Ta_1_1*	F:	GCCGATTTGTGTTCCGTAGT	~550	
		R:	TTGCGAAACCGATAGGAAAG		
Owm405		F:	CGATGGACTTCAAGCTCTCC	~600	PA
		R:	GCAAGAAGAAACTTCCATTTCG		
Owm406	*RAC875_c583_341*	F:	TCCGGACTGAGAACATTCCT	~500/1100	LP
		R:	TTCAGGCCCATCAGTGGT		
Owm407		F:	GCAAGCAAGAGGGTGTGAG	~450	SNP, 176 G/C
		R:	GACTAATCGCTGGGATGGAA		
Owm408		F:	TTGCTCGAAATCACATGGAA	~500	
		R:	AGCAATCAAACTGACAATAAATCTTCT		
Owm409	*BS00021920_51*	F:	CGGCATGACGAAGGAGTATC	~600	SNP, 365 G/A
		R:	TCGCCCTACACTGCTTTTCT		
Owm410		F:	TCTTGGCTGTTAGCACTTGAAA	~500	SNP, 194 T/C
		R:	GCGATCCACAGGAGTAGGAG		
Owm411		F:	CGCAGATCGTTGCCAATA	~650	PA
		R:	CAGGCAACGTTGGTTCTGTA		
Owm412		F:	AAGATCAAACCATACGAACACG	~500	PA
		R:	CGGTGGAAACTGTACTGCTG		
Owm413	*Tdurum_contig45539_226*	F:	AGTGTGCCTACCGAAAGCTG	~600	SNP, 147 T/G
		R:	GATCCACAGGGACACCTCAC		
Owm414	*BobWhite_c11006_801*	F:	ATCAAGTACCCGGTCTGAGC	~600	
		R:	GATCAGCAAAAGAGGGGATG		
Owm415		F:	GGATACGGTCTTTGCTGGAA	~600	
		R:	CGAGCAGCGGATACAACTG		
Owm416		F:	GGGCACGATATTGTTCGAGT	~500	
		R:	ATTGCAATCACTGCACAAGG		
Owm417		F:	CCACTGTGTTGCCTGTGTCT	~550	SNP, 369 C/A
		R:	TCCGTTCCAAAATACTTGTCG		
Owm418		F:	GATTTGTTGTGGCCTTCTTGA	~500	PA
		R:	TCTGGTAGAAAGGATGCATGG		

Marker	Based on SNP markers		Forward (F) and reverse (R) primer sequences (5′→3′)	Amplicon length [bp]	Polymorphism*
Owm419	*GENE-3346_506*	F:	GACATGAATCAAAAAGATATTCATCA	~500	PA
		R:	CAGTGACTCACCTGGAGATAGG		
Owm420	*RAC875_s118882_78*	F:	TTTGGTGTGGTTGTCCTTGA	~450	
		R:	ACCAAATCCACCAGCAACAT		
Owm421		F:	AGGGCTTGGATTTTTGGAGT	~500	LP/ SNP 200 T/C
		R:	AACCCAGGTAGGACCTTTGG		
Owm422	*BS00049977_51*	F:	GGGTCCCTGCAGTTAGTCTG	~500	SNP, 393 A/G
		R:	AATACCGCCACGAATGTGAT		
Owm423		F:	CCAAGTGCAACACCTTGCT	~500	SNP, 240 A/G
		R:	CAATGTCCGCAGAGTTCAAA		
Owm424		F:	GGAGGAGGAGGAGACTCAGG		
		R:	GAGACAGGCGATCGGAAATA		
Owm425	*Tdurum_contig47370_77*	F:	GATTCTCCAAACCCCACTGA	~450	
		R:	TGCAGTGGATTCAAAGCAAG		
Owm426		F:	TTCTTTCGGCTTCAAGGTTC	~500	SNP, 195 C/T
		R:	GGCAGAAAAATCATCCTTGG		
Owm427		F:	AGGAGTTGGGCTCATTTTTG	~500	
		R:	CACTAGTCACTGCCCAATGC		
Owm428	*D_contig26179_372*	F:	AGGGACGTTCTGAAACGATG	~500	
		R:	GGACAGCCAACAAAGGGATA		
Owm429	*RAC875_rep_c105803_144*	F:	GCTTCAAACTGCCAAAGAGG	~550	SNP, 201 T/C
		R:	AGCCAATTTCCCTTCTTCGT		
Owm430		F:	TCACAACAAACCGTGGAGAG	~450	
		R:	GCTGAACTGCATTTGAGTGG		
Owm431		F:	TCAAATGCAGTTCAGCCAAG	~450	PA
		R:	CTCCCTCCGTCCCATAATGA		
Owm432	*wsnp_CAP11_c923_558715*	F:	TGTCCAGTTTTCCAACCACA	~500/700	LP
		R:	CGACTGGAACGCTTGAATTT		
Owm433		F:	TCGCGAAATCTGTGAGTGAC	~450	SNP, 179 T/G
		R:	GATCCGTTGGCTGGAAGATA		
Owm434		F:	TGCTGGAACAAACGACTACG	~450	SNP, 349 C/T
		R:	TCAGGGTTTTAGGCATGTCA		
Owm435	*RAC875_c19313_887*	F:	ATCTGCAACGCTCTCAAGGT	~500	
		R:	GACGCAGGGTATCCATGTCT		
Owm436		F:	AGCCTCTCAAACTCGGACAA	~450	SNP, 213 C/G
		R:	TGACCAAACGTTGCATCAAT		
Owm437		F:	TCGGAGTGGGTTCTTTTGAC	~500	SNP, 414 T/C
		R:	GGATCAAACAAGCCCAGAAA		
Owm438		F:	TGCAATCCTGCTGATTCAAG	~500	SNP, 116 C/T
		R:	ACGAATGGCCTTTGATGATT		
Owm439	*wsnp_Ra_c25624_35192195*	F:	GATCGAGAAAGTCCCGAGTG	~500	
		R:	CCATTTGTCGGGAAGGTATG		
Owm440		F:	ATGTACCAGAGGACGGCAAC	~500/550	LP/ SNP, 188 A/G
		R:	GGTGGAGGCATATGGAAAAA		
Owm441	*wsnp_Ex_c3136_5798236*	F:	GAGAGGACTCCGCTGCAAT	~450	SNP, 93 C/T
		R:	GTGGAGTAGGGGAGGAGGTT		
Owm442		F:	CGACTGGTGTGGTTGTTGAC	~550	
		R:	TCGCAGAATGCTGACAGTTC		

Marker	Based on SNP markers		Forward (F) and reverse (R) primer sequences (5′→3′)	Amplicon length [bp]	Polymorphism*
Owm443	*Excalibur_rep_c68005_67*	F:	CAGGACATTATGCCAGTGTGA	~550	
		R:	ATGAGCGGACATAATGAAAGC		
Owm444		F:	GCAAGTGAAACTGCATCAGC	~500	
		R:	CTCCAACCCCCAACAAAGT		
Owm445		F:	CGTTGTAGGGGAAGCAGGTA	~500	SNP, 284 A/G
		R:	ATCGTGGAATGGTTCAAAGG		
Owm446		F:	CATCCGTAACGTGGTCATCA		
		R:	AATTTCGTATCAGCGGCATC		
Owm447	*Excalibur_c11797_118*	F:	GGGCCGATACCTCTCCTAAT	~550	
		R:	CCGTAGAGCACTGCGATAAA		
Owm448		F:	CTAGCCGCGAGTTATCCATC	~450	
		R:	CTCCCAATGTTTGTGTGGTG		

Appendix 7. *Owm449–Owm484* PCR markers.

[1] Position of a sequence in IWGSC RefSeq v1.0 (IWGSC, 2018) chromosome 3B (*Owm449–Owm466*) and 5A (*Owm467–Owm484*) that was used for BLASTN searches against the *T. monococcum* database (as described in Methods).

[2] LP – length polymorphism; PA – presence-absence polymorphism; SNP – single nucleotide polymorphism (the position of the SNPs is calculated from the beginning of the corresponding PCR products).

The empty cells in the last two columns mean either no PCR product or no polymorphism.

Marker	Positions of sequences using which the markers were designed [cM] [1]	Forward (F) and reverse (R) primer sequences (5′→3′)	Amplicon length [bp]	Polymorphism [2]
Owm449	662556233-662556965	F: GCCCAACTTTGGATTGTGTT R: CGACTACTGCGGCTTTTCTC	~800	SNP, 186 T/C
Owm450	665464444-665464936	F: GGTGGTGCAAGAGGATGAAT R: CAGAAGCCCATGAAGATGGT	~900	
Owm451	667778573-667779377	F: AAATCTTGCCATGCGTAACC R: TATGCCACTGCTCCAGTGAT	~850	
Owm452	668888263-668889679	F: CAGGAAGCTGACATGAACCA R: GTGCGCGGCTACTACTTCTC	~870	
Owm453	671017958-671018053	F: AAAGAAGCGCATACCACCAC R: ATCGTTGGCTACTCCATTGC	~850	SNP, 175 G/C
Owm454	672104337-672105914	F: CATTTCGTGTCCGATGTTTG R: GAGCGTGGGGTTTGTGTAGT		
Owm455	673849549-673850198	F: CCCGAGATGAGATCCTACCC R: GTTACGGAGGAGGAGGTGGT	~870	
Owm456	676207819-676208047	F: AGCCGAGATAAAGCAGACGA R: GCTTGACGGATGTTGGTTCT	~700	
Owm457	678441264-678442146	F: GAACTTGGACAGCACAAGCA R: ATCGCCCAGTCATAATCGTC	~870	SNP, 355 A/C
Owm458	680426706-680427059	F: TGGGTAACAGTCAGCGAGAA R: GACGGAGGGAGGGTTTTC		
Owm459	682786423-682787556	F: GTCCTCTTCTCCCTCCTGCT R: TGGCGGTACGGGATTACTAC	~900	SNP, 261 A/C
Owm460	683680118-683680559	F: ACACACCACTCTCACCCACA R: CATGTTGTCTGCAGCTTCGT	~1100	
Owm461	685037982-685039317	F: TCGATCTGATGGGGAGAAAC R: CAAAGGCCAATGACACCTCT	~1000	SNP, 92 G/C
Owm462	686337233-686337911	F: ACGAGGAAGGAGAGGAGGAC R: GCAGATCCATGGCAAAGACT	~1050/900	LP
Owm463	686346706-686348270	F: GAATAAACCGAACGCACAGG R: GGGAAGTTCTTCACGCACA	~850	PA

Marker	Positions of sequences using which the markers were designed [cM] [1]	Forward (F) and reverse (R) primer sequences (5′→3′)	Amplicon length [bp]	Polymorphism [2]
Owm464	687064081-687064593	F: ATGCTCCATCGGCTACCTC R: AAAGGGTTGCTTTCACATCG	~1000	
Owm465	688395988-688396412	F: CATCGTCCTCCCATGTTGAT R: ATTTTGGGCTGAGTGGAGTG	~1000	SNP, 190 C/G
Owm466	689517655-689517948	F: GCGTCAAGGATGAGAAGAGG R: AATGCTAACACACGCCACAA	~900	SNP, 164 G/T
Owm467	688007659-688008112	F: TCAGTCTTGTAATGTCGGTGCT R: CGGGGAAGGACGTAGAAATC	~850	SNP, 272 C/T
Owm468	688141827-688142338	F: GGGCAAGTTGAGCCTAAGTG R: GCAGTCTGCAATGAACCAAA	~1100	SNP, 385 T/C
Owm469	688301294-688301461	F: ACGCCTCATGATGGTAGGAC R: TCTTGTCGCAGTGTTCTTGG	~900	SNP, 146 T/C
Owm470	688663140-688664375	F: CAGTGCATACGCCAAGTCTG R: TGAACCCGACCTTATCTGCT	~850	SNP, 491 C/G
Owm471	688764148-688764280	F: ACAACAACAAGCAGCAGCAC R: GCGTAAATCACTGGGCAGAT	~950/1000	LP
Owm472	688946924-688950064	F: GTGTGCCCATCCCATTTATT R: GGAAAGGAGAATTCCCAAGC	~900	SNP, 303 C/T
Owm473	689386621-689387165	F: GGAAGCGTGACAGAGGCTAC R: CGTTATTAGTGGCCCATGCT	~850	
Owm474	689604449-689604667	F: AGCAGCTACCTGAAGCGAAA R: GCCCCTATGATTTCCAGTGA		
Owm475	689867453-689868092	F: CATCAGCCCGTTGGAGTC R: TGTGGCAGTGGTCTCATCAT	~850	
Owm476	689888876-689890310	F: CTGTCCACAGCAAGAGCAAA R: GCTCACTGGTCCATTCTTCG	~950/850	LP
Owm477	690188759-690191350	F: GGCTAGGCGATGAAGAACTG R: CACGCTAGCCACATCCCTAT	~900	
Owm478	690188759-690191350	F: CCAGTGTACAAGAGGGTGGAA R: GTGCGGCATGGCTATCTAAT	~900	PA
Owm479	690304923-690305295	F: CTACGGAGAGGCGATGTGTC R: GGAGGTGGTTGTGGACCTG	~950	
Owm480	690438536-690438887	F: GAGCAAGCTTCTTCGATTGG R: GGAAGTGAAGTCGGATCTGG	~1100/1500	LP
Owm481	690438536-690438887	F: CTCGACCAAGGATCTGGAAA R: GCGTCTGCCTCAGCTACTCT	~900	
Owm482	690566450-690566674	F: ATCTGAACGACTGGGAGCAC R: AGCAGGGAACTCCACATCAC	~1050	SNP, 509 T/C
Owm483	690944542-690944848	F: TGCCTCCTTCAAATCTCACC R: GCAAACGGGTACACGCTACT	~900	PA
Owm484	690951260-690953100	F: TAGTGGTGCACCGACATGAT R: TTCCTCGACTGAGGGAGCTA	~1050	SNP, 213 T/G