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**Exploring wheat wild relatives as a source of traits for drought
adaptation**

INAUGURAL DISSERTATION

Submitted in fulfillment of the requirements for the doctorate degree in agricultural
sciences (Dr. agr.)

to the Faculty of Agricultural Sciences, Nutritional Sciences and

Environmental Management

Justus Liebig University, Giessen

Submitted by

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Giessen, 2025

With permission by the

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This work was made possible with the support of a scholarship from the German Academic Exchange Service (DAAD).

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Abbreviations

<i>Ae.</i>	<i>Aegilops</i>
APX	Ascorbate peroxidase
AsA	Ascorbate
BM	Aboveground biomass
CWRs	Crop wild relatives
DHAR	Dehydroascorbate reductase
GR	Glutathione reductase
GSH	Glutathione
HI	Harvest index
MDHAR	Monodehydroascorbate reductase
PH	Plant height
POD	Guaiacol peroxidase
QTLs	Quantitative trait loci
RWC	Relative water content
ROS	Reactive oxygen species
SOD	Superoxide dismutase
<i>T.</i>	<i>Triticum</i>
TKW	Thousand kernel weight

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1 General introduction

1.1 Crop wild relatives

Crop wild relatives (CWRs) are closely related to domesticated crops (Maxted et al., 2006). Based on their degree of genetic relatedness and crossability, CWRs are categorized into three groups: primary gene pool, secondary gene pool, and tertiary gene pool (Maxted et al., 2006; Sharma et al., 2021). The primary gene pool consists of species that can easily hybridize with cultivated crop species. The secondary gene pool includes more distantly related species that have limited crossability and result in partially fertile hybrids. The tertiary gene pool comprises even more distant relatives, which require advanced techniques such as somatic hybridization for successful crossing and fertility recovery (Kashyap et al., 2022; Tadesse et al., 2019).

CWRs flourish in natural environments without human intervention and have adapted to harsh conditions through natural selection, making them resilient to various stresses (Kashyap et al., 2022; Maxted et al., 2006). Therefore, they possess vast genetic resources that play an important role in crop improvement programs (Dempewolf et al., 2017; Kilian et al., 2021). Since the beginning of crop domestication, CWRs have been continuously used to improve yield and nutritional quality of crops (Dempewolf et al., 2017; Palmgren & Shabala, 2024; Tanksley & McCouch, 1997). While most domesticated crops originate from wild species (Dempewolf et al., 2017; Maxted et al., 2006), the adaptive capacity and resilience of these wild species remain largely unexplored (McCouch, 2013).

1.1.1 Crop wild relatives for future food security

Global crop production relies on a few widely cultivated species, with wheat, rice, and maize providing over half of the world's daily protein and calorie needs (Foulkes et al., 2022). Moreover, just about a dozen species account for 80 % of total dietary intake (McCouch, 2013). With food demand expected to rise by 56 % by 2050 (van Dijk et al., 2021), increasing agricultural productivity, resilience, and sustainability is essential for ensuring food security (Farooq et al., 2024; McCouch, 2013). However, global food production faces challenges such as limited arable land, resource depletion (e.g., degradation of soil and water resources), population growth, and climate change (Reynolds & Braun, 2022). These threats to food security increase the risk of hunger and malnutrition, particularly in the developing world (Reynolds & Braun, 2022; van Dijk et al., 2021).

Crop domestication and breeding have significant contribution to today's food supply (Lei et al., 2024), yet they have also created genetic bottlenecks, reducing genetic diversity and increasing the risk of crop failure due to changing climatic conditions (Kilian et al., 2021;

Palmgren & Shabala, 2024; Tanksley & McCouch, 1997). Moreover, extensive use of breeding lines has narrowed genetic diversity in elite germplasms for further improvements (Allier et al., 2020; Lei et al., 2024). To counter this, breeders seek new genetic variations. CWRs provide significant genetic diversity (Flint-Garcia et al., 2023; Nevo & Chen, 2010). Breeders can enhance crop resilience by exploring and utilizing this diversity. Improving crop resilience is essential for sustainable agriculture and for mitigating the impacts of climate change on global food security (Hawkesford, 2024; Toulotte et al., 2022). CWR expand the germplasm base and provide opportunities to integrate new desirable traits (Leigh et al., 2022). Therefore, utilizing CWRs is a key strategy in plant breeding programs for developing climate-resilient cultivars (Palmgren & Shabala, 2024; Razzaq et al., 2021). Their genetic diversity boosts breeding programs (Castañeda-Álvarez et al., 2016; Leigh et al., 2022).

CWRs provide valuable traits such as resistance to biotic and abiotic stresses, enhanced flavor, and nutrition, thereby strengthening food resilience and diversity (Dempewolf et al., 2017; McCouch, 2013). Their genetic diversity also fosters new market opportunities and innovative products (Borelli et al., 2020). Additionally, the use of CWRs is marked by changes in land use and agricultural expansion (Renzi et al., 2022). CWRs thrive in marginal areas with minimal input and under extreme conditions (Renzi et al., 2022), making them well-suited for adapting to harsh and diverse environments (Dempewolf et al., 2017).

The use of CWRs in crop improvement was first identified by Vavilov in the early 20th century (Maxted et al., 2006), and since then, increasingly larger efforts have been made to utilize them in crop improvement programs (Dempewolf et al., 2017; McCouch, 2013; Palmgren & Shabala, 2024).

1.1.2 Crop wild relatives in crop improvement

Crop improvement programs have utilized CWRs to enhance genetic diversity and improve resistance to biotic and abiotic stresses (Dempewolf et al., 2017; Farooq et al., 2025; Hajjar & Hodgkin, 2007). Some examples of CWRs contributing disease resistance genes to crops include *Solanum demissum*, a wild potato, for late blight resistance in potatoes (Paluchowska et al., 2022); *Oryza rufipogon* Griff., a wild rice, for bacterial blast resistance in rice (Ballini et al., 2007); and *Triticum dicoccoides*, wild emmer wheat, for rust resistance in wheat (Yaniv et al., 2015). Similarly, CWRs serve as ideal sources for abiotic stress tolerance such as heat, drought and salinity (Farooq et al., 2025; Nevo & Chen, 2010; Palmgren & Shabala, 2024; Peleg et al., 2009). For instance, the introduction of the *TmHKT1;5-A* gene from diploid einkorn wheat (*Triticum monococcum* L.) has increased salt tolerance in wheat (Munns et al., 2012) and *DREBs* genes from wild emmer wheat have conferred drought tolerance in wheat (Lucas et al., 2011).

CWRs also hold significant potential for enhancing various traits in domesticated crops from yield to nutritional content (Leigh et al., 2022; McCouch, 2013). For instance, the wild tomato species (*Solanum pennellii*) has been successfully used to increase the yield of commercial tomatoes by over 50 % across diverse growing conditions (Gur & Zamir, 2004). Wild wheat species are particularly notable for their rich micronutrient contents like iron and zinc (Distelfeld et al., 2006; Tabbita et al., 2017; Zeibig et al., 2024b). Huge variations in grain nutrient concentrations have been found in CWRs of wheat (Zeibig et al., 2024b) and barley (Wiegmann et al., 2019), providing valuable sources for biofortification. Moreover, wild wheat species, known for enhancing protein and vitamin contents (Shewry & Hey, 2015), play crucial roles in improving wheat-based meat analogs as the demand for plant-based protein rises (Alcorta et al., 2021; Johansson et al., 2024). Growing awareness of health and dietary needs, along with changing food habits, is driving the demand to explore and utilize CWRs (Borelli et al., 2020; Farooq et al., 2025; Johansson et al., 2024). Furthermore, with advancements in modern breeding tools and increasing knowledge about their benefits, the use of CWRs is expected to rise in the future (Castañeda-Álvarez et al., 2016; Flint-Garcia et al., 2023). Therefore, it is crucial that these valuable genetic resources are conserved and explored for future use.

1.2 Wheat domestication

Wheat (*Triticum* spp.) has been an important food crop throughout agricultural history and remains crucial to global food security. It is one of the most widely and successfully cultivated crops worldwide, contributing around 19 % of calories and 20 % of proteins in the human diet (Langridge et al., 2022; Shiferaw et al., 2013; Tadesse et al., 2019). Modern wheat production is dominated by two species; *Triticum aestivum* L. (BBAADD, bread or common wheat) and *Triticum durum* Desf. (BBAA, durum or pasta wheat), accounting for approximately 95 % and 5 % of the total global wheat production, respectively (Sharma et al., 2021; Tadesse et al., 2019).

The evolutionary history of wheat is characterized by successive allopolyploidization events involving ancestral diploid species of *Triticum* and *Aegilops*, leading to the emergence of tetraploid and hexaploid wheat species (Figure 1) (Kilian et al., 2010; Zeibig et al., 2022). Therefore, the genus *Triticum* exhibits diverse taxa, classified as diploid, tetraploid, and hexaploid based on their ploidy levels (Zeibig et al., 2024a). Furthermore, only four naturally occurring wild species of *Triticum* have been identified: two diploid species, *Triticum urartu* Thumanjan ex Gandilyan ($2n = 2x = 14$, AA) and *Triticum boeoticum* Boiss. ($2n = 2x = 14$, AA), known as wild einkorn; and two tetraploid species, *Triticum dicoccoides* (Körn. ex Asch. & Graebner Schweinf.) ($2n = 4x = 28$, BBA^uA^u, wild emmer) and *Triticum araraticum* Jakubz. ($2n = 4x = 28$, GGA^tA^t) (Sharma et al., 2021; Zeibig et al., 2022).

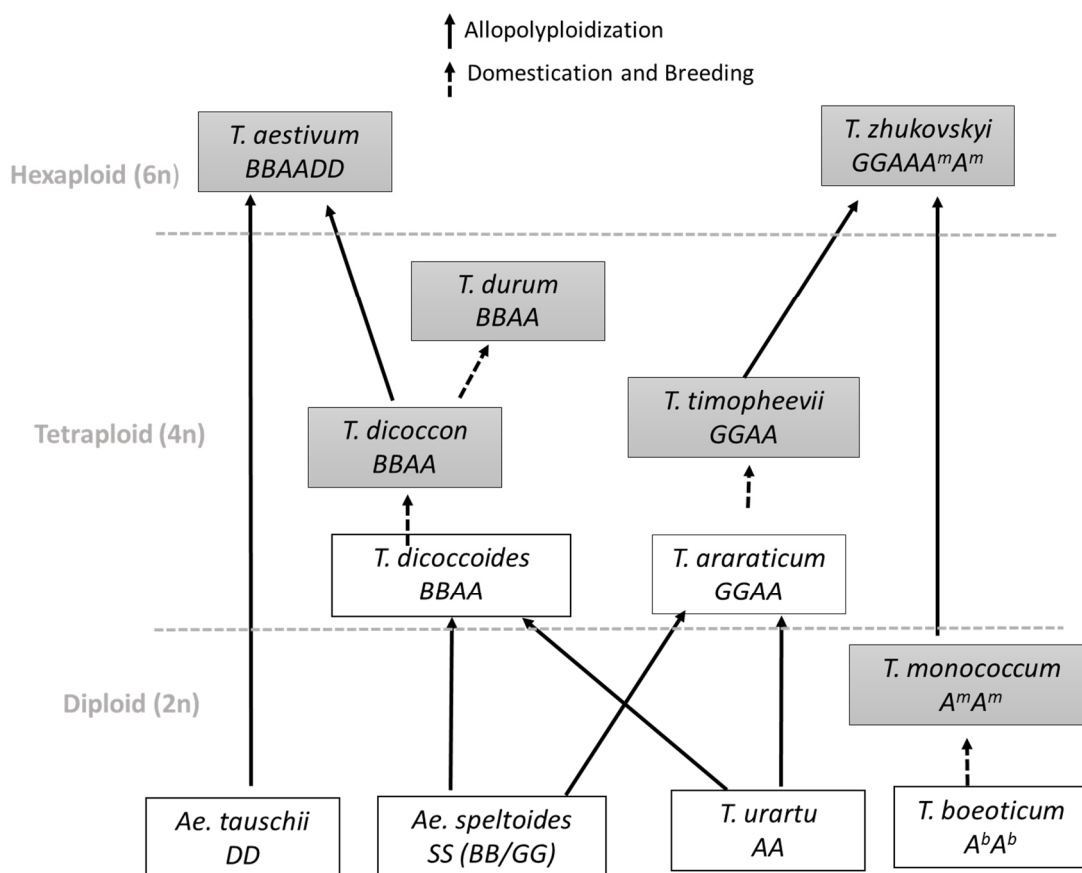


Figure 1: The evolution and domestication of wheat (modified from Kilian et al., 2010). Wild species are shown in white text boxes and domesticated wheats are shown in grey text boxes. The continuous black lines stand for allopolyploidization, while black dashed lines indicate domestication and breeding. *Triticum* taxa grouped as diploid (2n), tetraploid (4n) and hexaploid (6n) are separated by grey dashed lines.

Wheat was first domesticated around 10,000 years ago during the Neolithic Revolution in the Fertile Crescent of the Near East (Feldman & Levy, 2023). The primary goal of domestication was to adapt its cultivation for more efficient and easier human use. This process began with the harvesting of wild wheat, eventually progressing to the cultivation of landraces and the development of modern cultivars (Feldman & Levy, 2023; Sharma et al., 2021). Wild wheat relatives possess survival traits such as seed shattering, small seeds, hulled grains and awns that promote seed burial; however these traits hinder harvesting and threshing (Zeibig et al., 2024a). The domestication process involved the selection of desirable traits like large, hull-less seeds with free-threshing characteristics and non-shattering spikes, which improved harvestability and benefited yield (Sharma et al., 2021;

Zeibig et al., 2022, 2024a). This selection of desirable traits has evolved through both conscious and unconscious processes, enhancing the crop's suitability for cultivation and harvesting (Flint-Garcia et al., 2023; Renzi et al., 2022).

1.3 Drought stress and adaptive mechanisms

Drought stress is one of the major abiotic stresses affecting crop production (Toulotte et al., 2022). Currently, about 40 % of the world's arable land area is classified as dryland (arid, semi-arid and dry sub-humid) (Pal et al., 2023), with drought conditions affecting more than 70 countries (UNCCD, 2019). Future predictions indicate that drought-related yield losses for major crops will increase by more than 50 % by 2050, further threatening global food security (Palmgren & Shabala, 2024). Drought stress limits the crop growth and development from germination to maturity, reducing nutrient uptake, photosynthesis, and ultimately affecting grain yield (Farooq et al., 2024; Sallam et al., 2019). It disrupts photosynthesis, consequently producing excess reactive oxygen species (ROS), which leads to oxidative damage to nucleic acids, lipids, and proteins (Abid et al., 2018; Suneja, et al., 2017).

Plants evolve mechanisms to perceive environmental stimuli and cope with drought stress through changes in morphological, physiological, biochemical and molecular processes (Farooq et al., 2009; Sallam et al., 2019). They enhance water absorption through their improved root systems, reduce stomatal opening to limit water loss, and regulate biochemical processes to optimize carbon resources in response to initial drought (Zia et al., 2021). As the drought continues, stomatal regulation, photosynthesis, osmotic balance, and antioxidant defence mechanisms are altered to withstand drought stress (Farooq et al., 2009; Zia et al., 2021). The response to drought stress depends on the severity, duration, intensity of the drought, as well as the plant's species, genotype, and growth stages (Sallam et al., 2019). In general, plants develop three drought adaptation strategies to cope with drought stress: drought escape, drought avoidance, and drought tolerance (Sallam et al., 2019; Zia et al., 2021). Plants escape drought by accelerating their life cycle, such as by early maturation, or avoid it by minimizing water loss through mechanisms like stomatal closure, reducing leaf size, leaf curling, and developing deep roots (Li et al., 2017; Sallam et al., 2019). Additionally, they can tolerate drought by enhancing antioxidant activity and undergoing osmotic adjustment, which involves the accumulation of compounds like proline and total soluble sugars (Izanloo et al., 2008; Sallam et al., 2019). Drought tolerance is a complex process, so plants employ a mixed approach to adaptation; therefore, drought adaptation strategies are not mutually exclusive (Farooq et al., 2009; Shavrukov et al., 2017).

1.3.1 Drought adaptive traits in wild wheat relatives

While domestication and breeding have gradually eliminated negative traits over time, prioritizing grain yield (Lei et al., 2024; Voss-Fels et al., 2019), they have also led to the loss of genetic diversity (Dempewolf et al., 2017). Moreover, the domestication process has reduced genetic variations in physiological traits like photosynthesis, stomatal functions and root traits, which are vital for drought adaptation in plants (Leigh et al., 2022; Placido et al., 2013). CWRs possess specific traits that can be adapted to the adverse environmental conditions (Dempewolf et al., 2017), offering significant genetic diversity for drought tolerance (Mehrabad Pour-Benab et al., 2019; Nevo & Chen, 2010; Peleg et al., 2009; Suneja et al., 2019).

Wild wheat relatives exhibit great variations in photosynthetic traits, including electron transport, maximum rates of carboxylation and Rubisco activity (Leigh et al., 2022; McAusland et al., 2020). Notably, wild emmer wheat has wider genetic diversity, containing genes and quantitative trait loci (QTLs) linked to drought tolerance (Aberkane et al., 2021; Peleg et al., 2009), and demonstrates high water use efficiency (Peleg et al., 2005). Other wild wheat species such as *T. boeoticum*, *Ae. tauschii* and *Ae. crassa* have high expression level of antioxidant-related genes (Mehrabad Pour-Benab et al., 2019). Introgression lines from wild wheats enhance yield, biomass, and water stress resilience in cultivated wheat by improving gas exchange, carbon assimilation, and root growth (Merchuk-Ovnat et al., 2016; Placido et al., 2013). Additionally, genetic resources from wild wheats and landraces further enhance drought adaptation through the accumulation of soluble carbohydrates (Reynolds et al., 2007). Primitive landraces such as *Triticum monococcum* L., *Triticum dicoccum* (Schrank) Schuebl., and *Triticum spelta* L. have high chlorophyll content, which contributes to drought tolerance (Konvalina et al., 2014). On the other hand, some researchers argue that crop domestication and breeding unintentionally enhanced traits like photosynthesis and nitrogen use, boosting crop yield and productivity (Lei et al., 2024; Voss-Fels et al., 2019). Consequently, modern wheats are suitable for cultivation in both high- and low-input environments due to improved water efficiency, photosynthesis, and enhanced adaptability (Voss-Fels et al., 2019). Nevertheless, wild wheat relatives and landraces serve as a valuable genetic reservoir for drought tolerance, with further exploration needed to aid future wheat breeding programs (Leigh et al., 2022; Nevo & Chen, 2010; Pour-Aboughadareh et al., 2021).

1.4 Scope and aims

Wild wheat relatives offer valuable genetic resources for drought tolerance. While modern wheat cultivars have achieved high yield potential through selective breeding (Voss-Fels et al., 2019), this process has narrowed genetic diversity, creating a bottleneck that may

reduce their resilience to environmental stresses, including drought (Dempewolf et al., 2017; Toulotte et al., 2022). To secure future wheat production in the face of climate change, especially in marginal growing areas, it is important to explore the resilience and genetic variation of wild wheat relatives (Peleg et al., 2009; Reynolds et al., 2007). Consequently, there is growing interest in investigating drought-tolerant characteristics in wild wheat relatives (Leigh et al., 2022). However, their morphological and physiological adaptations to drought stress remain largely unexplored. The main aim of this thesis was to explore drought adaptation traits of wild wheat relatives within the *Triticum* taxa, assess their agromorphological and physiological responses to drought stress, and identify the underlying tolerance mechanisms.

To fulfill this aim, a comprehensive investigation into drought tolerance traits within an expanded gene pool of *Triticum*, including the secondary gene pool (GGAA), was performed through agromorphological and physiological characterization. In the first step, a field experiment was carried out, which involved the screening of a large set of *Triticum* accessions, and the subsequent field evaluation of selected genotypes for their morphological and physiological responses to drought stress. Chapter 2 presents the findings that form the foundation for selecting genotypes based on their relative yield performance under drought stress. Moreover, it highlights the findings on the drought stress response of the selected genotypes, analyzing both morphological and physiological traits under rainfed and irrigated conditions across two seasons in the field. Furthermore, Chapter 3 presents the findings of a greenhouse experiment that assessed the physiological and biochemical responses of selected genotypes to drought stress imposed at two critical stages: first, during stem elongation, and subsequently, during anthesis. In both the field and greenhouse experiments, a genotype-dependent response within the *Triticum* gene pool was observed. These findings also revealed significant intra- and interspecific variations in physiological and morphological traits within the *Triticum* genus. Potential drought-tolerant candidates were also identified. Subsequently, the objective of Chapter 4 was to compare potential drought-tolerant genotypes of the neglected species *T. araraticum* with modern bread wheat cultivars to identify underlying drought-adaptive mechanisms in *T. araraticum*. The result from Chapter 4 revealed a unique antioxidant response in *T. araraticum* that is not present in modern bread wheat cultivars. Overall, the insights from this dissertation highlight the drought adaptation traits of wild wheat relatives and landraces, offering promising drought-tolerant candidate genotypes for future wheat breeding programs.

2 A comparison of drought responses in wild wheat relatives and domesticated wheat grown under irrigated and rainfed field conditions

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Published in: *Field Crops Research*, 321 (2025) 109678

DOI: 10.1016/j.fcr.2024.109678



Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr



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ARTICLE INFO

Keywords:

drought tolerance
Triticum
stomatal conductance
grain yield
isohydric
anisohydric

ABSTRACT

Context: Domestication and breeding processes for developing modern wheat plants from diverse wild relatives and landraces have had unintended effects of loss of genetic diversity. This reduction in genetic variation undermines the ability of modern wheat cultivars to tolerate environmental stresses such as drought. Wheat wild relatives possess untapped genetic potential for tolerating abiotic stress, especially drought. Yet, their morpho-physiological adaptation and drought stress resilience mechanisms remain underexplored.

Objective: This study aimed to investigate the adaptive responses of plants within the *Triticum* spp. gene pool, encompassing wheat wild relatives, landraces, and modern cultivars to drought stress under rainfed and irrigated field conditions.

Methods: From an initial pool of 110 genotypes screened during the first growing season in 2022, 20 best performing genotypes, including modern cultivars for comparison, were selected for a second growing season in 2023 based on their relative yield performance. Two different treatment conditions, irrigated and rainfed, were applied during both growing seasons. This experiment observed single plants per replicate. Multiple parameters, including days to heading and flowering, plant height, number of spikes per plant, spike length, spike weight per plant, straw weight per plant, aboveground biomass per plant, grain yield per plant, thousand kernel weight, harvest index, stomatal conductance, and vegetation indices, were assessed on the selected genotypes.

Results: Taking averages measured across both growing seasons, we observed significant genotypic variation across several parameters: days to heading and flowering, plant height, number of spikes per plant, spike length, spike and straw weight per plant, aboveground biomass per plant, grain yield per plant, thousand kernel weight, harvest index, stomatal conductance, and vegetation indices. Water stress during the rainfed treatment significantly reduced grain yield (by 21 %) and stomatal conductance (by 45 %). Stomatal conductance was associated with grain yield and yield-related traits under rainfed conditions. Diverse physiological drought tolerance mechanisms associated with stomatal regulation were identified, revealing genotype-specific responses to drought stress. Genotypes such as *T. dicoccoides* (G242), *T. urartu* (G45), *T. boeoticum* (G27) and *T. araraticum* (G221) exhibited isohydric adaptation, whereas *T. monococcum* *sinskajae* (G89) and *T. durum* cv. Sambadur (G41) exhibited anisohydric adaptation.

Conclusion: Some genotypes of *T. dicoccoides*, *T. urartu*, *T. boeoticum* and *T. araraticum* exhibited isohydric adaptation, while *T. monococcum* *sinskajae* and *T. durum* cv. Sambadur exhibited anisohydric adaptation under drought stress which needs further verification. These genotypes can serve as donors for introducing drought tolerance traits within wheat improvement programs.

Abbreviations: ARI2, anthocyanin reflectance indices; BM, aboveground biomass per plant; CR11, carotenoid reflectance index 1; DTF, days to flowering; DTH, days to heading; SPP, number of spikes per plant; gsw, stomatal conductance; GY, grain yield per plant; HI, harvest index; Lic2, Lichtenthaler index 2; NDVI, normalized difference vegetation index; PCA, principal component analysis; PH, plant height; PRI, photochemical reflectance index; SIPI, structure insensitive pigment index; SL, spike length; Spwt, spike weight per plant; Stwt, straw weight per plant; TKW, thousand kernel weight; PhipS2, photosystem II efficiency.

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<https://doi.org/10.1016/j.fcr.2024.109678>

Received 25 March 2024; Received in revised form 14 November 2024; Accepted 20 November 2024

Available online 29 November 2024

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Implications: These findings holds great significance in improving drought tolerance in modern wheat breeding programs.

1. Introduction

Drought is a major abiotic stress that threatens crop productivity worldwide (IPCC, 2023), with climate change causing more severe and unpredictable droughts that adversely affect plant growth (Munné-Bosch and Villadangos, 2023). Drought stress affects plants in complex ways (Badaeva et al., 2022; Khadka et al., 2020; Nevo and Chen, 2010), which vary according to their phenological stage as well as the intensity and duration of drought stress (Chaouachi et al., 2023; Mohammadi, 2018). Wheat requirements for satisfying the food demands of a growing global population will inevitably increase (Bapela et al., 2022; Khadka et al., 2020; OECD-FAO, 2020). Environmental stresses may also constrain production (Kulkarni et al., 2017). This issue could be resolved by breeding cultivars with enhanced drought tolerance. Therefore, an understanding of the mechanisms underlying drought tolerance and the development of well-adapted, drought-tolerant varieties are critical needs.

Common wheat (*Triticum aestivum* L., $2n = 6x = 42$, BBAADD) is a major staple food crop for underpinning global food security, contributing approximately 20 % of total dietary calories in human nutrition (Shiferaw et al., 2013). Recent studies have explored the complexities of wheat evolution and taxonomy (Feldman and Levy, 2023; Sharma et al., 2021; Zeibig et al., 2022). Only four naturally occurring wild *Triticum* species have been found: *Triticum urartu* Thumanjan ex Gandilyan ($2n = 2x = 14$, AA), *T. boeoticum* Boiss. ($2n = 2x = 14$, AA, wild einkorn) and two wild tetraploid species, *T. dicoccoides* (Körn. ex. Asch. & Graebner) Schweinf. ($2n = 4x = 28$, BBA⁴A⁴, wild emmer), and *T. araraticum* Jakubz. ($2n = 4x = 28$, GGA⁴A⁴).

Genetic diversity has been reduced within many breeding pools through crop domestication and improvement, resulting in a genetic bottleneck (Dempewolf et al., 2017). Moreover, the loss of genetic diversity in cultivated varieties has limited their ability to withstand various environmental (biotic and abiotic) stresses (Nevo and Chen, 2010; Peng et al., 2011; Sharma et al., 2021). Wheat wild relatives provide important genetic source material in modern wheat breeding programs (Kilian et al., 2021; Leigh et al., 2022). They are well-adapted to harsh environments and contain potentially useful alleles that can improve drought tolerance in wheat cultivars (Ahmadi et al., 2018; Nevo and Chen, 2010). Some studies that have evaluated agronomical and physiological traits associated with drought tolerance in wild emmer wheat *T. dicoccoides*, and have identified certain genotypes as potential donors for introducing drought tolerance traits (Budak et al., 2013; Peng et al., 2011; Suneja et al., 2019; Zhang and Kirkham, 1994). By contrast, *T. araraticum* (GGA⁴A⁴) has received less attention despite its potential value in plant breeding (Badaeva et al., 2022). Sultan et al. (2012) found that it is less tolerant to drought than other species, but their study only examined one accession of *T. araraticum* under artificially imposed drought conditions at the early seedling stage. Other taxa, such as *T. urartu* and *T. boeoticum* (Ahmadi et al., 2018; Pour-Aboughadareh et al., 2017) as well as *T. monococcum*, *T. carthlicum*, *T. dicoccon*, and *T. durum* (Zhang and Kirkham, 1994), exhibit drought tolerance primarily via antioxidant defense mechanisms at the seedling stage.

When exposed to drought stress, plants exhibit both short-term and long-term responses (Oguz et al., 2022). Short-term responses to drought stress include changes in stomatal conductance, water potential, turgor pressure, and biochemical composition. The long-term response is the cumulative effect of short-term effects, which include changes in plant morphology, phenology, and grain yield caused by disruption of the plant's entire physiology (Oguz et al., 2022). Adaptive mechanisms under drought stress include maintenance of stomatal

conductance and retention of photosynthetic pigments (Aspinwall et al., 2015; Kulkarni et al., 2017; Suneja et al., 2019). Stomatal regulation in wheat varies between isohydric (water-saving) and anisohydric (water-wasting) drought adaptation strategies (Guizani et al., 2023). According to the prevailing conceptual model explaining isohydric and anisohydric strategies, the degree of stomata closure is a response to water stress (Martínez-Vilalta and García-Fórner, 2017). Isohydric plants minimize water loss and can maintain leaf water potential under water stress conditions by rapidly reducing stomatal conductance. By contrast, anisohydric plants keep their stomata open and increase transpiration in response to water stress and maintain their photosynthetic rate (Bandurska, 2022). An anisohydric strategy supports growth and development during short-term drought, whereas an isohydric response prioritizes passive survival during prolonged drought conditions (Martínez-Vilalta and García-Fórner, 2017; Sade et al., 2012). Understanding drought-related adaptive phenotypic traits that contribute to yield is a first and fundamental step toward advancing understanding of complex physiological and biochemical mechanisms (Mwadingeni, et al., 2016) and constitutes the basis for selecting drought-tolerant wheat genotypes (Reynolds et al., 2009; Sallam et al., 2019).

Most studies on drought tolerance in wheat wild relatives have been limited to examining plant physiology within a few taxa or samples per taxon during the seedling stage, without considering the effect of drought stress on grain yield. Moreover, there is a paucity of studies that have used wheat wild relatives under field conditions. Furthermore, these studies have not attended to the morphological and physiological traits contributing to yield-related traits. Therefore, we investigated the genetic variation underlying drought adaptation within a diverse collection of wheat wild relatives, landraces, and modern wheat cultivars under field conditions through an analysis of morphological and physiological traits associated with yield components. Accordingly, we conducted field experiments in a drought-prone location in Germany under two different moisture conditions: irrigated and rainfed. We tested the following hypotheses: (a) large variations in morphological and physiological traits exist within the *Triticum* gene pool; (b) these variations in morphology and physiology contribute to variations in drought tolerance; and (c) wheat wild relatives demonstrate greater drought tolerance than modern cultivars. The findings of this study will benefit future breeding programs aimed at developing drought-tolerant wheat cultivars in arid regions.

2. Materials and methods

2.1. Plant material

During the 2022 growing season, 110 wild and domesticated wheat (*Triticum*) genotypes were used in a screening experiment. They comprised 60 genotypes from 4 wild taxa, 44 genotypes from 20 landrace taxa, and 6 modern winter wheat varieties (Table S1). The wheat species were classified according to the classification system outlined in a previous study (Zeibig et al., 2024b), with slight modification. The modern winter wheat cultivars included in the study were 4 bread wheat cultivars: cv. Apostel (I.G. Pflanzenzucht), cv. Julius (KWS SAAT SE & Co.), cv. Nordkap (SAATEN UNION), and cv. RGT Reform (RAGT Saaten) and 2 durum wheat cultivars: cv. Sambadur (Hauptsaaten) and cv. Wintergold (SAATEN UNION). Twenty genotypes were selected for further testing during the 2023 growing season according to the results of the screening experiment conducted during the 2022 growing season. These genotypes belonged to 11 taxa comprising 4 wild taxa (11

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genotypes), 6 landrace taxa (one genotype each), and 3 modern cultivars (two of bread wheat and one of durum wheat). The selection of genotypes for the 2023 growing season was primarily based on their relative grain yield performance during the 2022 growing season (Table S2).

2.2. Experimental conditions and treatments

Field experiments were conducted during two winter wheat growing seasons (October 2021–June 2022 and October 2022–June 2023) in the sandy loam soil of the Gross Gerau experimental station at Justus Liebig University Giessen in Germany (49°56'N, 8°30'E; 92 m above sea level). The field capacity of the soil at the experimental location was classified as very low (0–130 mm / 100 cm) according to the classification of the Ministry of Nature Protection, Environment and Geology of the State of Hesse (HLNUG, 2024). Data on precipitation, temperature, and relative humidity were collected daily during the growing seasons from November to June (Figs. 1A and 1B). The total precipitation amounts during the 2022 and 2023 growing seasons were 281 mm and 294 mm, respectively. The minimum and maximum average temperatures were -1.8°C and 24.4°C , respectively, and the average relative humidity was 74 % during the 2022 growing season. In 2023, the minimum and maximum average temperatures were -3.4°C and 23.4°C , respectively, and the average relative humidity was 76 %. The maximum temperatures were 37°C in 2022 and 38°C in 2023, both were recorded at the end of June during the physiological maturity of the crop.

During both growing seasons, the seedlings were germinated using the procedure described by Zeibig et al. (2024a). Briefly, eight single seeds per genotype were sown in Quickpot trays and kept in a vernalization chamber for 48 hours at 5°C and at a relative humidity of 60 %–70 %. They were subsequently transferred to a greenhouse with a 10 h:14 h light/dark photoperiod and day/night temperatures of $15^{\circ}\text{C}/10^{\circ}\text{C}$. Lastly, they were acclimatized outdoors for two days before being transplanted into the field. Seeds used for the 2022 growing

season were sown on October 5, 2021 and subsequently transplanted on November 8, 2021. Seeds used for the 2023 growing season were sown on October 4, 2022 and transplanted on November 1, 2022. The plant-to-plant and row-to-row distances of transplanted seedlings were 75 cm and 1 m, respectively. The topsoil was sandy and loose, with hard rocks below 45 cm, making it prone to drought stress. Therefore, wider spacing was used to prevent plants from competing for water at shallow depth. A gap of 2.5 m was maintained between the irrigated and rainfed plots to prevent any seepage of irrigation water to the rainfed plots.

A randomized split plot experimental design was used with four replications (a single plant per replicate) under two treatment conditions: rainfed and irrigated during both growing seasons. Throughout the winter, no irrigation was applied for the rainfed or irrigated treatments. Only the irrigated plot received supplemental irrigation, and the water stress conditions were solely rainfed throughout the cropping seasons. Irrigation was applied using an automated overhead sprinkler. The plants in the irrigated plots were watered four times during both growing seasons between April 20, 2022 and June 8, 2022 and between May 26, 2023 and June 16, 2023. Irrigation ensured that water limitations did not constrain normal crop performance in the irrigated plots, considering the precipitation pattern. During the 2022 growing season, the irrigated plots received a total of 130 mm of supplemental irrigation at the jointing stage when the plants reached stage 33 of Zadoks growth scale (Z33; Zadoks et al., 1974), the heading (Z49), flowering (Z65), and grain-filling (Z80) stages. During the 2023 growing season, 83 mm of supplemental irrigation, in total, was applied at the flowering (Z61), late flowering (Z69), early grain-filling (Z77), and dough (Z85) stages of growth.

Soil moisture was measured using an HH2 moisture meter, version 4.2 (Delta-T Devices Ltd., Cambridge, UK) after irrigation commenced. The measurements were taken at different soil depths of approximately 6 cm and 30 cm at a randomly selected location in each replication for both treatment conditions. After the onset of irrigation, they were taken

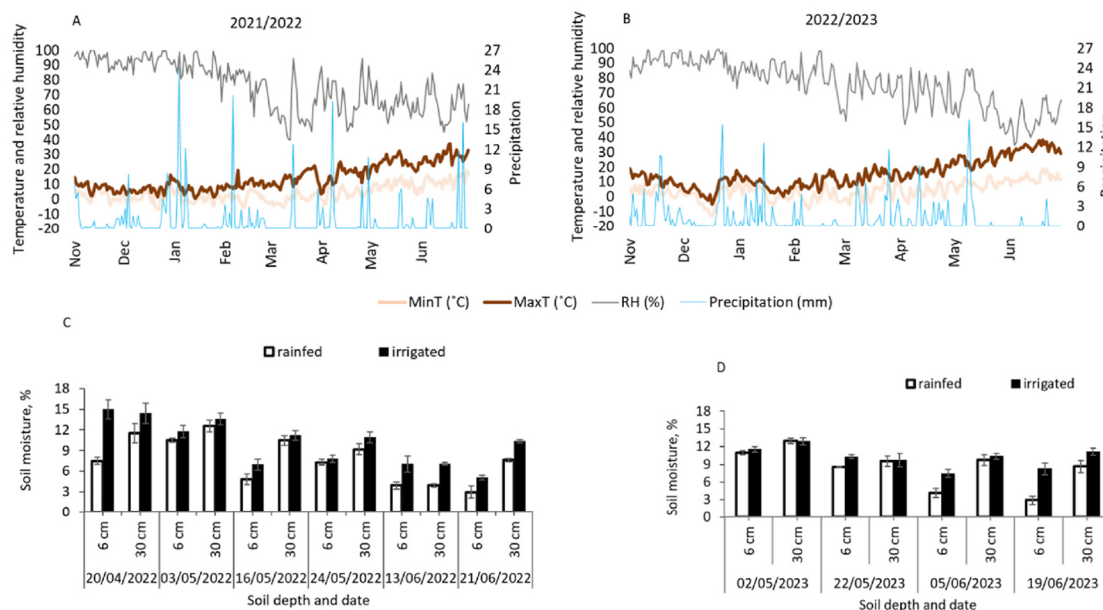


Fig. 1. Weather data, and soil moisture status during the crop growing periods at the research station in Gross Gerau, Germany. Average daily temperatures, precipitation, and humidity levels during the 2022 growing season (A) and the 2023 growing season (B). The percentage of soil moisture was measured on different dates at different soil depths during the 2022 growing season (C) and the 2023 growing season (D). The soil moisture under irrigated and rainfed conditions are depicted as black and white bars, respectively, and the values are means \pm standard errors ($n=4$).

on different dates during the crop season. For both growing seasons, all the plants were harvested by the second week of July when they were fully matured.

2.3. Trait measurements

A total of 19 traits were investigated during the 2022 and 2023 growing seasons. These traits covered several categories. Two phenological traits were measured: days to heading (DTH) and days to flowering (DTF). Nine of the traits were morphological and yield-related: plant height (PH), number of spikes per plant (SPP), spike length (SL), spike weight per plant (Spwt), straw weight per plant (Stwt), above-ground biomass per plant (BM), grain yield per plant (GY), thousand kernel weight (TKW) and harvest index (HI). The remaining eight traits were physiological: stomatal conductance (gsw), photosystem II efficiency (PhiPS2), normalized difference vegetation index (NDVI), Lichenthaler index 2 (Lic2), photochemical reflectance index (PRI), anthocyanin reflectance indices (ARI2), structure insensitive pigment index (SIPI), and carotenoid reflectance index 1 (CRI1). During the 2022 growing season, three replicates were used for measuring physiological traits, whereas all other measurements were obtained using four replicates. However, during the 2023 growing season, four replications were included for all of the above measurements.

During both growing seasons, phenological data (DTH and DTF) were recorded following the Zadoks growth scale (Zadoks et al., 1974) as soon as the individual plant reached Z50 and Z60, respectively. Phenological traits were recorded with reference to the seed sowing date for the respective phenological stages. PH was measured post-flowering from the soil surface to the tip of the spike, excluding the awns. SL was measured from the base to the tip of the spike, excluding the awns. The main tiller of the plant was considered for both phenological and morphological trait measurements. SPP were counted during the physiological maturity period (Z90). Single plants for each genotype were harvested when fully mature. BM was determined after the harvested samples were oven dried at 30 °C to attain a constant weight. The spikes were threshed using an electric coffee mill. GY per plant and TKW were subsequently measured. The HI for each plant was calculated by dividing GY per plant by BM per plant.

Spectral reflectance measurements (vegetation indices) were recorded using a Polypen RP 410 portable instrument (Photon Systems Instruments, Drasov, Czech Republic). Of the many vegetation indices described in the literature, the following were selected: NDVI = $(R780 - R630)/(R780 + R630)$; Lic2 = $R440/R690$ (Begum et al., 2020); PRI = $(R531 - R570)/(R531 + R570)$ (Sims and Gamon, 2002); ARI2 (anthocyanin reflectance indices) = $R790(1/R550 - 1/R700)$ (Gitelson and Merzlyak, 1997); CRI1 = $1/R510 - 1/R700$ (Gitelson et al., 2003); and SIPI = $(R790 - R450)/(R790 - R650)$ (Peñuelas et al., 1995). A portable handheld LI-600 porometer system integrated with a fluorometer (LI-COR, Lincoln, Nebraska, USA) was used to measure gsw and PhiPS2 between 10:00 h and 14:00 h on a sunny day. All of these physiological traits were measured at the flowering stage (Z60), after clipping the middle portion of the leaf below the flag leaf of the main tiller. We used the leaf below the flag leaf for measurements because the flag leaf of some species was too narrow for the LI-600 sensor.

2.4. Data analysis

To rank the genotypes for drought tolerance, a “relative value” was calculated for each genotype as described by Bouslama and Schapaugh (1984) but with slight modifications. The relative value was calculated as the ratio of the rainfed value of the individual genotype to the average irrigated value of the same genotype for each studied trait. Relative values provide an effective way to rank morphologically diverse genotypes, as the stress levels were interpreted relative to their initial conditions. The statistical analysis was performed in R version 4.2.0 (www.r-project.org). The effects of genotype and treatment and their

interaction were obtained from the model fitted with lmer from the lme4 package (Kuznetsova et al., 2017), with genotype and treatment treated as fixed factors; year and replication by treatment by year interaction were treated as a random effects. Multiple test comparisons were performed with the emmeans function. Principal component analysis (PCA) was performed using FactoMineR and Factoextra in R. To obtain Pearson's correlation, the Hmisc package was used in R. Figures were generated with ggplot2 and Microsoft Excel.

3. Results

3.1. Soil moisture under irrigated and rainfed conditions

During the respective growing seasons in 2022 and 2023, the rainfed treatment area received approximately 31 % and 22 % less water than the irrigated treatment area, which benefited from supplemental irrigation. Therefore, relative to the irrigated condition, the soil moisture was reduced under the rainfed condition, decreasing on average by 25 % and 20 %, respectively, during the growing seasons in 2022 (Fig. 1C) and 2023 (Fig. 1D).

3.2. Genotypic variations exist in the Triticum gene pool under both irrigated and rainfed conditions

We investigated genotypic variation in 110 genotypes during the 2022 growing season, mainly considering two yield-related traits: BM and GY (Table 1). Significant genotypic differences in BM and GY were detected in wild wheats and landraces under both irrigated and rainfed conditions. A significant genotypic effect on GY in modern cultivars only occurred under rainfed conditions.

The relative GY values ranged between 0.17 and 2.12 for wild wheats, 0.32 and 3.00 for landraces, and 0.40 and 1.53 for modern cultivars, averaged across all genotypes within a genetic group during the 2022 growing season. The 110 genotypes were ranked from 1 to 110 according to the average relative BM and GY values, where a rank value of 1 indicated the most drought-tolerant genotype, and a rank value of 110 indicated the most drought-sensitive genotype (Table S2). We observed highly significant treatment effects on both BM and GY for all genetic groups when averaged across all genotypes during the 2022 growing season (Table 1). However, the genotype-by-treatment effect was significant for both BM and GY in the landrace group but not in wild wheats and modern cultivars. The average values of phenological, morphological, and yield-related traits for 110 genotypes grown under irrigated and rainfed conditions during the 2022 growing season are shown in Table S6.

3.3. Effects of drought stress on physiological, morphological, and yield traits

We evaluated morphological, physiological, and yield-related traits in 20 genotypes over two growing seasons in 2022 and 2023 (Table 2). We intentionally selected genotypes of wild relatives and landraces that exhibited high relative performance based on GY and BM in the screening experiment conducted in 2022 (Table S2) to compare them with contemporary modern cultivars. We then analysed the physiological parameters of those genotypes across two seasons. The average values decreased for all the measured traits except PhiPS2 under rainfed conditions compared with those grown under irrigated conditions due to water stress, when averaged across 20 genotypes over two growing seasons (Table 2, S3 and S4). Water stress caused significant reductions in gsw, GY, Spwt, SL, NDVI, PRI, ARI2, CRI1, and SIPI under rainfed conditions. Notably, gsw and GY decreased by more than 20 %, whereas other traits decreased by less than 20 %. The effect of genotype was highly significant for GY and all yield-related traits. However, among the yield-related traits, only GY was significantly affected by the treatment-by-genotype interaction. All the physiological traits apart

Table 1
Performances of 110 genotypes from different genetic groups under irrigated and rainfed conditions during the 2022 growing season.

	Wild			Landrace			Modern cultivar					
	df	Irrig.	Rain	Rv	df	Irrig.	Rain	Rv	df	Irrig.	Rain	Rv
Aboveground biomass per plant (BM), g												
Minimum		15.57	10.98	0.25		33.69	18.37	0.39		62.27	30.24	0.41
Maximum		93.01	66.77	1.46		210.90	223.33	1.80		130.19	108.28	1.39
Mcan		47.59	31.38	0.67		101.45	73.42	0.75		85.73	68.15	0.85
Standard error		1.94	1.41	0.03		4.77	3.82	0.04		12.14	8.69	0.04
Genotype	59	**	***		43	***	***		5	ns	ns	
Treatment	1		**		1		***		1		***	
Genotype: Treatment	59		ns		43		*		5		ns	
Grain yield per plant (GY), g												
Minimum		3.67	2.18	0.17		5.32	5.78	0.32		30.44	13.14	0.40
Maximum		24.33	15.66	2.12		83.07	88.92	3.00		64.74	50.31	1.53
Mean		10.86	6.93	0.67		35.76	25.31	0.75		41.24	32.84	0.87
Standard error		0.49	0.35	0.03		1.87	1.58	0.05		6.19	4.88	0.14
Genotype		*	*			***	***			ns	*	
Treatment	1		***		1		***		1		***	
Genotype: Treatment	59		ns		43		*		5		ns	
Treatment												

Notes: Irrig. = irrigated, Rain = rainfed, df = degree of freedom, Rv= relative value calculated as the ratio of rainfed to the mean value for the irrigated condition for individual plants. *, **, and *** denote significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns = nonsignificant.

from PhiPS2 also exhibited highly significant genotypic differences, and the genotype-by-treatment interaction was significant for gsw, PhiPS2, SIPI, Lic2, PRI, and CRI1. Moreover, the genotype effect, but not the treatment or the genotype-by-treatment interaction effects on DTH, DTF, and PH were highly significant.

3.4. Genotypic responses to drought stress in the Triticum gene pool

We compared treatment effects on individual genotypes using data for 20 genotypes averaged over two seasons in 2022 and 2023. *T. dicoccon* (G198) and *T. compactum* (G213) showed significant decreases in BM (Fig. 2A). The treatment significantly affected GY in *T. dicoccon* (G198), *T. compactum* (G213), *T. aestivum* (G10), *T. aestivum* cv. Apostel (G134), and *T. aestivum* cv. Nordkap (G181) (Fig. 2B). BM and GY measurements revealed that *T. durum* cv. Sambadur (G41) had the highest water stress tolerance, followed by *T. dicoccoides* (G242), *T. monococtum sinkajae* (G89), *T. araraticum* (G221), *T. urartu* (G45), and *T. boeoticum* (G27), each with an average relative value of 1. The treatment effect on HI or TKW was non-significant for any of the genotypes (Figs. 2C and 2D). However, *T. dicoccon* (G198), *T. compactum* (G213), and *T. aestivum* cv. Nordkap (G181) exhibited significant decreases in Spwt induced by the treatment (Figure S1A), with the greatest relative changes observed in *T. durum* cv. Sambadur (G41) and *T. dicoccoides* (G242), each with an average relative value of 1. Treatment effects on Stwt were significant for *T. dicoccon* (G198), *T. compactum* (G213) and *T. aestivum* (G10) (Figure S1B). The highest relative Stwt values were observed for *T. urartu* (G45) and *T. dicoccoides* (G90), each with an average relative value of 1.8.

Most of the genotypes from wild *T. boeoticum* (G27 and G33), *T. urartu* (G45), and *T. dicoccoides* (G242 and G248), as well as the landrace, *T. carthlicum* (G159), showed a significant decrease in stomatal conductance due to water stress (Fig. 3A). However, *T. spelta* (G109) and *T. araraticum* (G189) exhibited low stomatal responses. NDVI significantly decreased for *T. araraticum* (G161) and *T. araraticum* (G205) (wild), *T. aestivum* (G10) (a landrace) and *T. durum* cv. Sambadur (G41) due to water stress. By contrast, *T. compactum* (G213) and *T. carthlicum* (G159), had the highest average relative value of 1 (Fig. 3C). All the genotypes belonging to the landrace group except those in *T. aestivum* (G10) and all three modern cultivars, namely *T. aestivum* and *T. durum* taxa showed an increase in Lic2 under rainfed conditions relative to irrigated conditions. However, water stress caused a decrease in Lic2 in all the genotypes of the wheat wild relatives (Fig. 3D).

The genotype effect on DTH (Fig. 4A) was only significant for

T. urartu (G45), with none of the other genotypes exerting significant effects on DTF or PH (Figs. 4B and 4C). SPP decreased significantly in *T. aestivum* (G10) (Fig. 4D), and *T. urartu* (G45) had the highest relative SPP, followed by *T. durum* cv. Sambadur (G41). The treatment effect on SL was significant for *T. boeoticum* (G27), while *T. dicoccoides* (G242) and *T. araraticum* (G205) had the highest relative value of 1 (Figure S1C).

3.5. Correlation analysis under irrigated and rainfed conditions

A correlation analysis was performed on the 19 traits measured under irrigated (Fig. 5A) and rainfed conditions (Fig. 5B) over two seasons, including those for the dataset of 20 genotypes. Under both irrigated and rainfed conditions, yield-related traits, namely Spwt, Stwt, BM, GY, HI, and TKW exhibited significant positive correlations with each other, with the exception of Stwt with HI under both conditions and with TKW under rainfed conditions. PH was significantly negatively correlated with HI and TKW under irrigated conditions and with GY, HI, and TKW under rainfed conditions. There were significant positive correlations of DTH and DTF with Stwt, Spwt, BM, and GY under rainfed conditions. Under irrigated conditions, DTF was significantly correlated with Stwt and BM but not with Spwt or GY.

Strikingly, under rainfed conditions, gsw was significantly positively correlated with most yield-related traits. However, no such correlation was found under irrigated conditions. Significant positive correlations were observed between gsw and GY and between HI and TKW. There was no strong correlation between the NDVI and yield-related traits under either irrigated or rainfed conditions, with few exceptions. The NDVI was significantly positively correlated with Stwt under rainfed conditions and significantly negatively correlated with GY and TKW under irrigated conditions. CRI1 and SIPI were negatively correlated with yield-related traits. CRI1 was significantly negatively correlated with Spwt, BM, GY, HI, and TKW under irrigated conditions and with GY, HI, and TKW under rainfed conditions. Similarly, there were significant negative correlations between the SIPI and Spwt, GY, HI, and TKW under both irrigated and rainfed conditions.

3.6. Principal component analysis

PCA was also performed to examine the relationships between morphological, physiological, and yield-related traits and the diversity of genotypes under irrigated and rainfed conditions. The first five PCs explained 88.6 % of the total variation, and their eigenvalues were > 1

Table 2
Summary statistics for 20 genotypes averaged over two growing seasons in 2022 and 2023.

Traits	Mean Squares			Treatment Means (Standard Deviation)	
	Treatment	Genotype	Treatment × Genotype	Irrigated	Rainfed
	df: 1	df: 19	df: 19		
Days to heading	0.9 ^{ns}	552 ^{***}	11.4 ^{ns}	220 (8.9)	220 (9.8)
Days to flowering	2.7 ^{ns}	634 ^{***}	11.6 ^{ns}	229 (10.5)	228 (11.6)
Plant height (cm)	876 ^{ns}	3500 ^{***}	122 ^{ns}	99 (21.3)	95 (21.6)
Number of spikes per plant	57 ^{ns}	1409 ^{***}	68 ^{ns}	23 (13.8)	22 (13.6)
Spike length (cm)	16 ^{ns}	78 ^{***}	2.5 ^{ns}	10.4 (3.0)	9.6 (2.3)
Spike weight per plant (g)	1306 ^{***}	2753 ^{***}	439 ^{ns}	38.2 (25.7)	32.3 (20.3)
Straw weight per plant (g)	713 ^{ns}	2281 ^{***}	214 ^{ns}	30.5 (20.4)	26.0 (17.3)
Aboveground biomass per plant (g)	39134 ^{ns}	8891 ^{***}	1221 ^{ns}	68.7 (44.6)	58.1 (35.7)
Grain yield per plant (g)	914 ^{ns}	2179 ^{***}	269 ^{ns}	22.3 (19.4)	17.5 (14.1)
Thousand kernel weight (g)	59.5 ^{ns}	1533 ^{***}	14.7 ^{ns}	30.0 (10.7)	28.9 (10.48)
Harvest index	0.002 ^{ns}	0.13 ^{***}	0.004 ^{ns}	0.29 (0.10)	0.28 (0.12)
Stomatal conductance (mmol m ⁻² s ⁻¹)	77297 ^{***}	2951 ^{ns}	2400 ^{ns}	103 (52.7)	57 (30.5)
Photosystem II efficiency	0.001 ^{ns}	0.016 ^{ns}	0.028 ^{ns}	0.42 (0.16)	0.43 (0.16)
Normalized difference vegetation index	0.143 ^{***}	0.015 ^{***}	0.007 ^{ns}	0.57 (0.06)	0.52 (0.10)
Lichtenthaler index 2	0.023 ^{ns}	0.239 ^{***}	0.026 ^{***}	0.86 (0.13)	0.85 (0.21)
Photochemical reflectance index	0.002 ^{***}	0.002 ^{***}	0.0004 ^{ns}	-0.007 (0.016)	-0.01 (0.022)
Anthocyanin reflectance indices	0.55 ^{ns}	0.52 ^{***}	0.07 ^{ns}	-0.05 (0.23)	0.06 (0.43)
Carotenoid reflectance index 1	4.28 ^{**}	4.77 ^{***}	0.62 ^{**}	2.53 (0.77)	2.19 (0.91)
Structure insensitive pigment index	0.096 ^{***}	0.028 ^{***}	0.004 ^{ns}	0.62 (0.05)	0.58 (0.08)

Notes: Mean values are shown for irrigated and rainfed conditions and mean square values of all the investigated traits between treatments, genotypes, and genotype-by-treatment interactions. The values inside parentheses denote standard deviations associated with the corresponding mean values.

^{ns}p < 0.05

^{**}p < 0.01

^{***}p < 0.001

ns = nonsignificant.

under irrigated conditions (Table S5). Additionally, under rainfed conditions, the first four PCs had eigenvalues > 1 and explained 88.5 % of the total variation (Table S5). Moreover, under rainfed conditions, the first two PCs contributed 67.1 % of the total variation, with PC1 explaining 44.6 % of the variation and PC2 explaining 22.5 % of the variation (Figure S2B). Of the traits examined, the major contributors to these two PCs were HI, TKW, GY, DTH, DTF, Stwt, NDVI, SIPI, CR1, PH, and ARI2. The first two PCs explained 61.9 % of the variation (PC1: 41.9 % and PC2: 20 %) under irrigated conditions (Figure S2A). The

major contributing traits in these two PCs were GY, TKW, CR1, SIPI, NDVI, DTF, DTH, BM, and Spwt.

The genotype distribution determined via PCA revealed distinct clustering patterns under rainfed and irrigated conditions (Figure S2). All the genotypes of *T. dicoccoides* and *T. araraticum* (wild tetraploid species) were clustered together under the irrigation condition, while the genotypes of *T. boeoticum* and *T. urartu* (wild diploid species) occupied unique positions. Conversely, under rainfed conditions, *T. boeoticum* was dispersed from wild tetraploids. Notably, *T. urartu* held an intermediate position between *T. dicoccoides* and *T. araraticum* (the wild tetraploids) and *T. boeoticum* (the wild diploid). Landrace taxa and modern cultivars formed distinct clusters under both conditions, with the exception of diploid landraces of *T. monococcum* and the tetraploid landrace of *T. compactum*, which were more closely related to *T. boeoticum* and modern cultivars, respectively.

4. Discussion

4.1. Impact of water stress on grain yield

In this study, we evaluated the water stress response in the *Triticum* spp. gene pool, initially in a diverse set of 110 genotypes grown in 2022 (Table S1) and subsequently in 20 genotypes selected for a second growth season in 2023 (Table S2). Most of the *Triticum* taxa used in this study have already shown good adaptability to the Central European climate (Zeibig et al., 2024a). Among the yield components, GY was the most affected trait (Table 2). Our finding of a 21 % average decrease in GY is aligned with the findings of previous studies (Farooq et al., 2014; Wang et al., 2017), in which the GY of wheat decreased by 19 % and 28 %, respectively, during - drought stress. Our results fell within the range reported in these studies, indicating that plants experienced drought stress at critical reproductive stages, such as heading, flowering, and grain filling, which occurred between May and June in our study. Essentially, the reproductive stage is a crucial period, and water stress during this phase could result in a severe reduction in GY by affecting pollination and fertilization and causing inadequate grain filling (Farooq et al., 2014).

4.2. Genotypic variation in morpho-physiological traits within the *Triticum* gene pool

Our first hypothesis was that there is wide variation in morphological and physiological traits within the *Triticum* gene pool. To test this hypothesis, we measured various agro-morphological and physiological traits. Our results clearly showed significant genotypic differences (p < 0.001) in the *Triticum* taxa assessed using morphological, physiological (except for PhiPS2), GY, and yield-related traits (Tables 1 and 2). Previous studies have also reported such variation for a number of morphological and physiological traits within the *Triticum* gene pool (Reynolds et al., 2007; Suneja et al., 2017; Wang et al., 2017). This finding highlights that *Triticum* gene pool provides a valuable repository of genetic diversity, which could potentially be used to improve drought tolerance in elite cultivars within breeding programs. However, no variation in PhiPS2 might indicate that this trait is highly conserved and is likely more dependent on environmental conditions, which requires further confirmation. However, the genotype-by-treatment effect was significant in this study.

4.3. Morpho-physiological responses to water stress and their impact on yield traits

The second hypothesis in this study was that variations in drought tolerance occur within the *Triticum* gene pool. To test this hypothesis, we examined the impact of drought on agro-morphological and physiological traits to elucidate diverse drought stress responses among the evaluated genotypes. Previous studies have shown that drought stress

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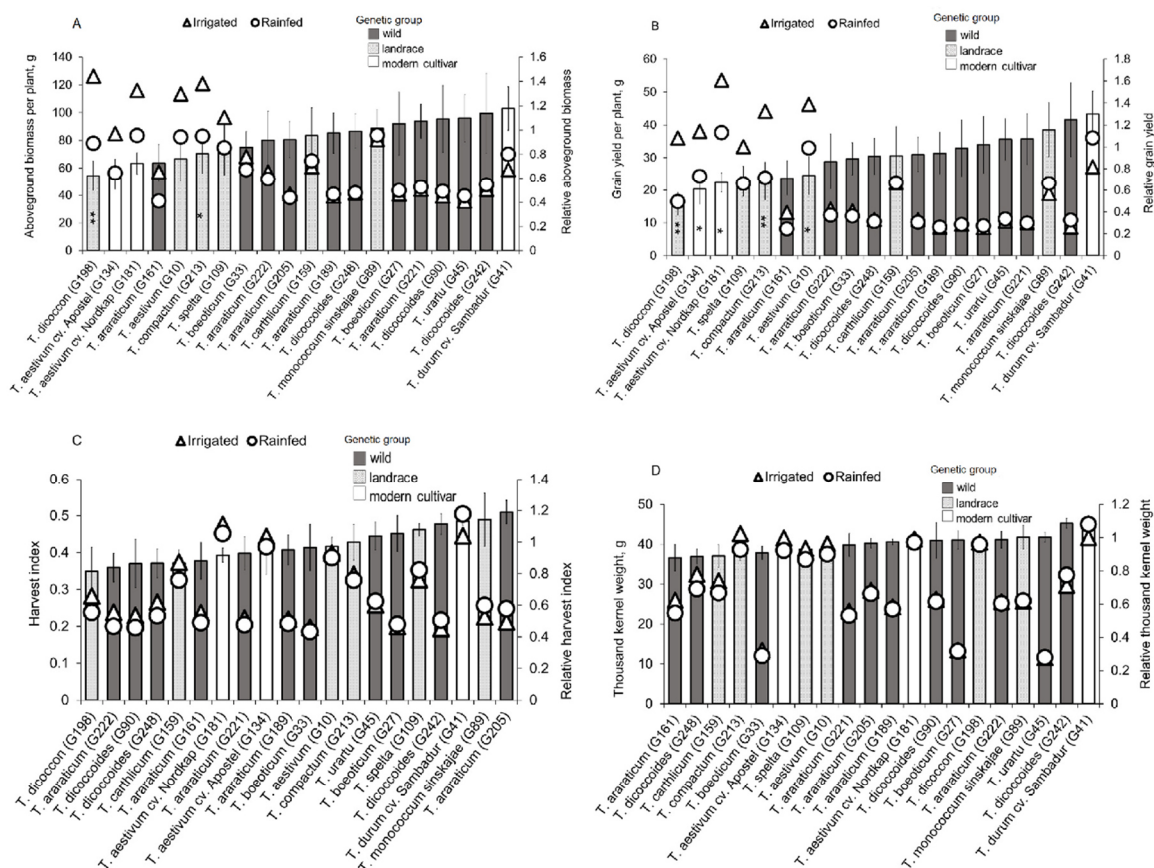


Fig. 2. Effect of drought stress on yield-related traits in 20 wheat genotypes. Aboveground biomass per plant (A), grain yield per plant (B), harvest index (C) and thousand kernel weight (D). The primary vertical axis depicts the means under rainfed and irrigated conditions. The data are presented as average values across two growing seasons in 2022 and 2023. Open circles and triangles indicate means under rainfed and irrigated conditions, respectively. The asterisks within the vertical bar indicate significant differences between rainfed and irrigated conditions (treatment effect) for the same genotype (* p < 0.05; ** p < 0.01; *** p < 0.001). Mean relative values ± standard errors of the means are depicted on the secondary vertical axis represented by bars. The genotypes are arranged in ascending order from low relative values (left) to high relative value (right). The different shades indicate the genotypes' genetic groups.

significantly decreases GY (Shokat et al., 2023; Wang et al., 2017). They support our finding that water stress significantly affects GY and other yield-related traits, such as Spwt and SL (Table 2). The results of our correlation analysis and PCA (Fig. 5 and S3) revealed a positive correlation between GY and other yield-related traits, such as Spwt, BM, HI, and TKW that was consistent across irrigated and rainfed conditions. This finding endorsed the previous study (Zhang et al., 2019), which reported a positive correlation between GY and yield-related traits such as TKW. Yield traits are important components for enhancing GY in wheat (Qaseem et al., 2019), however their usually low heritability might pose a challenge for plant breeders (Chen et al., 2012).

The significant genotype-by-treatment interaction effects observed across multiple traits, especially gsw and GY in this study reveals the diverse responses among genotypes under water stress (Table 2). Similar interactions were reported for wheat in previous drought stress studies (Lopes et al., 2012; Suneja et al., 2017). This finding highlights the need to consider specific genotypes for optimizing crop yields, especially under diverse environmental conditions. Drought stress did not affect GY in certain genotypes from different genetic groups. These were *T. boeoticum* (G27), *T. urartu* (G45), *T. dicoccoides* (G242), and *T. araraticum* (G221) from the wild group; *T. monocooccum* *sinskajae* (G89) from the landrace group; and *T. durum* cv. Sambadur (G41) from

the modern cultivar group, which also exhibited high relative values for yield-related traits, such as BM, Spwt, Stwt, and HI. Therefore, these genotypes showed greater tolerance in terms of yield traits than other genotypes. Conversely, compared with other genotypes, those from *T. dicoccon*, *T. compactum*, and *T. aestivum* (G10) and the modern bread wheat cultivars, *T. aestivum* cv. Apostel (G134), Nordkap (G181) showed lower tolerance with significant and greater decreases in GY and other yield traits, such as BM, Spwt, and Stwt. Genotypes from taxa such as *T. boeoticum*, *T. urartu*, *T. dicoccoides*, *T. monocooccum*, and *T. durum*, have previously been reported to be drought tolerant (Aberkane et al., 2021; Ahmadi et al., 2018; Suneja et al., 2017; Zhang and Kirkham, 1994).

4.4. Drought stress adaptation: differential stomatal regulation in the *Triticum* gene pool

The gsw trait enables stomata to control the exchange of gases, primarily water vapor and carbon dioxide between the plant and its surroundings (McAusland et al., 2020). Stomatal closure is one of the first responses to drought stress in plants and strongly influences the characteristics associated with photosynthesis and transpiration (Li et al., 2017). A previous study reported a significant positive correlation

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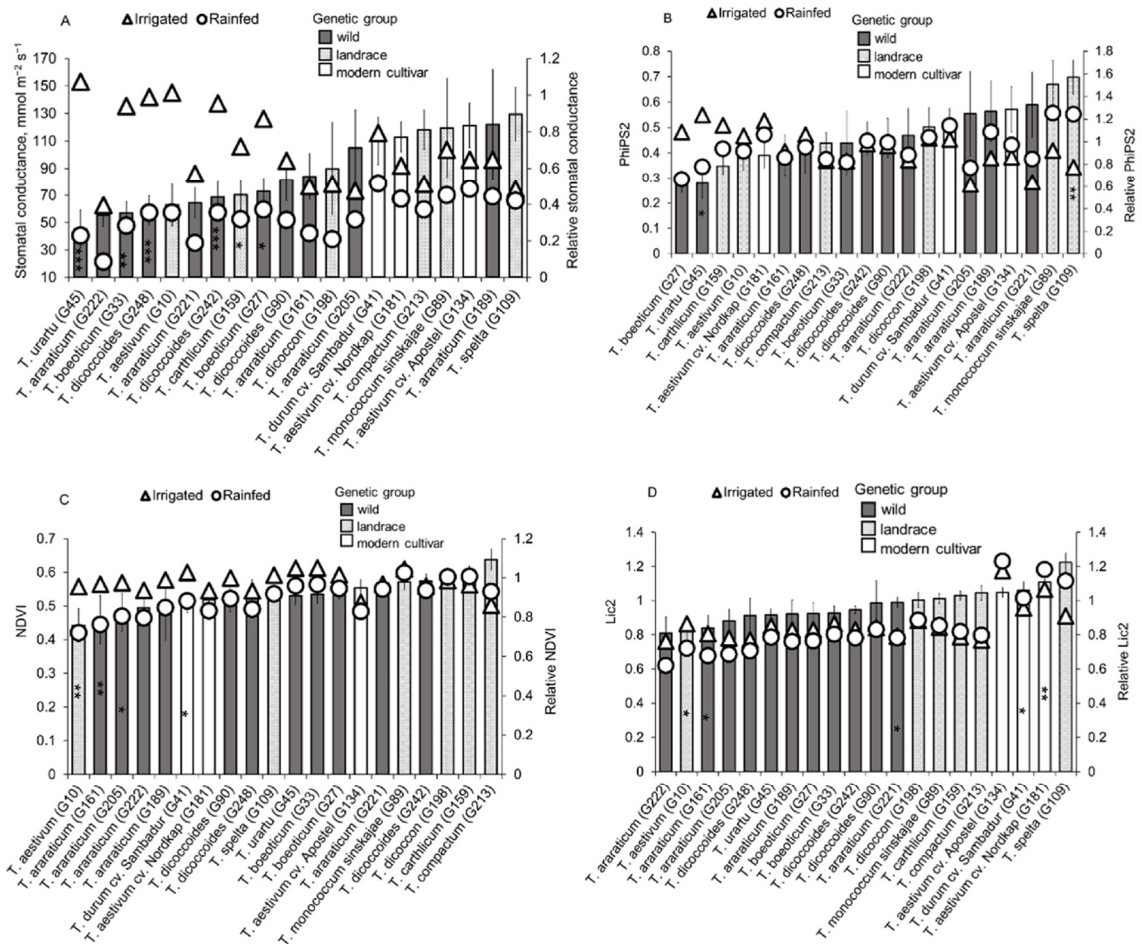


Fig. 3. Effect of drought stress on physiological traits in 20 wheat genotypes; stomatal conductance (A), PhiPS2 (B), NDVI (C) and Lic2 (D). The primary vertical axis represents the means under rainfed and irrigated conditions. Open circles and triangles indicate means under rainfed and irrigated conditions respectively. The data are presented as average values over two growing seasons in 2022 and 2023. Mean relative values \pm standard errors of the means are shown on the secondary vertical axis represented by bars. The genotypes are arranged in ascending order from low relative values (left) to high relative value (right). The different shades indicates the genotypes' genetic groups. The asterisks inside the vertical bar indicate significant differences arising from the treatment effect (rainfed and irrigated conditions) for the same genotype (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). PhiPS2 = photosystem II efficiency, NDVI = normalized difference vegetation index, Lic2 = Lichtenthaler index 2.

between gsw and GY under both irrigated and water stress conditions (Aminian et al., 2011). In contrast, we observed a significant positive correlation between gsw and GY and yield-related traits only under water stress conditions.

In our study, a significant reduction in gsw occurred due to water stress (Table 2). This finding is consistent with those of previous studies (Pour-Aboughadareh et al., 2017; Saeidi and Abdoli, 2015), which reported a significant decrease in gsw caused by drought stress. Notably, at the species level, gsw reduction in *T. urartu* was 74 %, that was highest among all species included in our study, which contradicts the findings of Pour-Aboughadareh et al. (2017), who reported a 26 % lower reduction in gsw in *T. urartu* at the seedling stage compared with gsw reduction in other wild wheats and in *T. aestivum*. These contrasting results could be explained by dynamic, stage-dependent developmental responses of gsw to moisture stress within *Triticum* taxa, indicating the complexity of their adaptive mechanisms.

A significantly greater reduction in gsw was observed in species such as *T. urartu*, *T. dicoccoides*, and *T. boeoticum*, despite lower reductions in

yield traits. The most tolerant genotypes with an isohydric adaptive mechanism were *T. dicoccoides* (G242), *T. urartu* (G45), *T. boeoticum* (G27), and *T. araraticum* (G221), whose GY and yield-related traits were maintained but whose gsw reduction exceeded the average gsw reduction. This process can be an isohydric adaptive mechanism for maintaining yield traits through efficient water management entailing stomata closure. Isohydric wheat genotypes prioritize water preservation, leading to reduced photosynthesis and subsequent decreases in grain yield (Guizani et al., 2023). However, efficient water management directly enhances resilience to drought stress, ensuring a consistent yield even in unpredictable climatic conditions, which could be associated with stomatal morphology (Bertolino et al., 2019). Therefore, the finding of a large reduction in gsw in some wild wheat genotypes in this study could indicate improved water use efficiency through the maintenance of water potential, which requires further verification. However, not all the genotypes of the wild species exhibited isohydric adaptation, which suggests that adaptation in wheat is genotype specific rather than species dependent, as proposed in a previous study

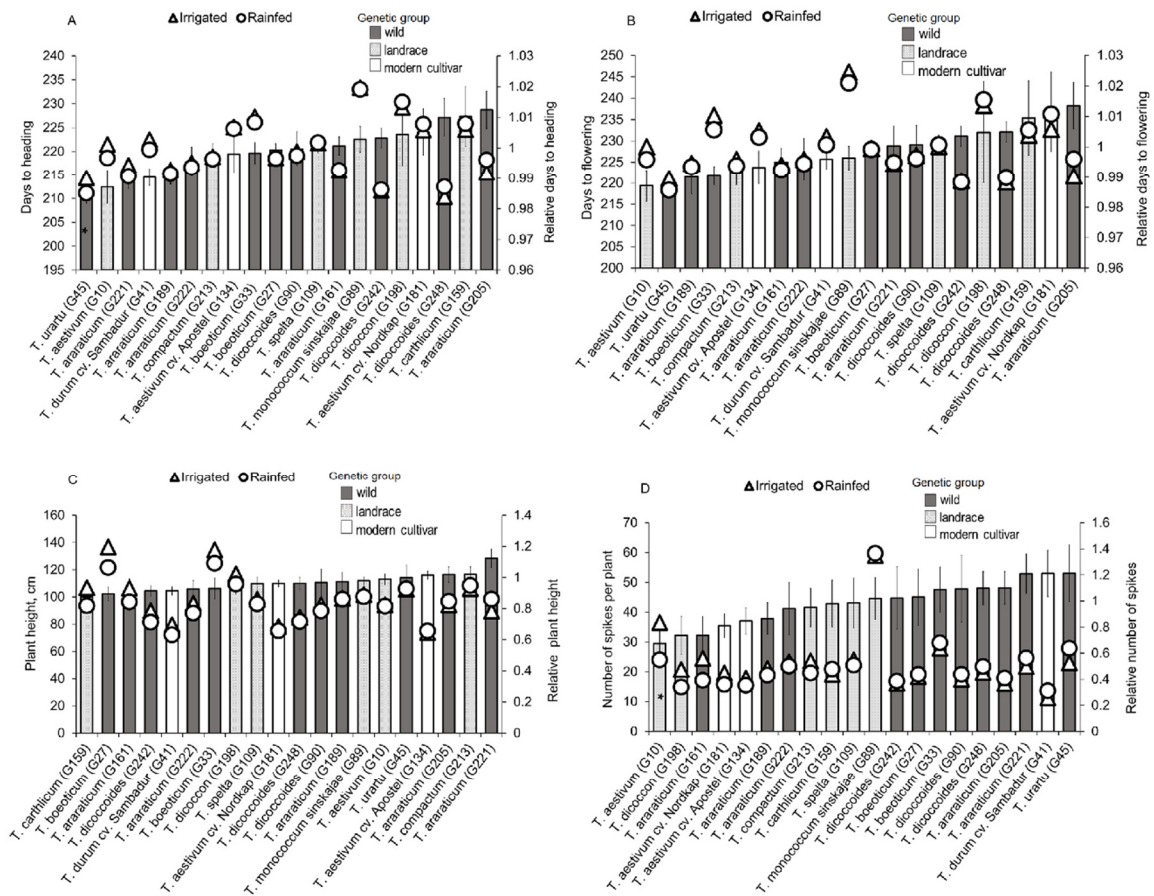


Fig. 4. Effect of drought stress on phenological and morphological traits in 20 wheat genotypes; days to heading (A), days to flowering (B), plant height (C) and number of spikes per plant (D). The primary vertical axis shows the means under rainfed and irrigated conditions. Open circles and triangles indicate means under rainfed and irrigated conditions, respectively. The data are presented as average values over two growing seasons in 2022 and 2023. The mean relative value \pm standard errors of the means are shown on the secondary vertical axis presented by bars. The genotypes are arranged in ascending order from low relative values (left) to high relative value (right). The different shades denote the genetic groups of the genotypes. The asterisks inside the vertical bar indicate significant differences arising from the treatment effect (rainfed and irrigated conditions) for the same genotype. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(Onyemaobi et al., 2021).

The most tolerant genotypes from the landrace group, *T. monococcum* *sinskajae* (G89), and the modern durum wheat cultivar, *T. durum* cv. Sambadur (G41), exhibited anisohydric behavior, with a decline in gsw below the average, with maintenance of GY and yield traits in line with those reported in previous studies (Guizani et al., 2023; Onyemaobi et al., 2021). This indicates drought-tolerant wheat genotypes deploying anisohydric adaptation to maintain yield traits through osmotic adjustment. Both isohydric and anisohydric strategies are beneficial for plants under drought stress conditions (Bandurska, 2022) and they can be linked with the root morphology (Saradadevi et al., 2014). Isohydric wheat genotypes might be associated with root signals for stomatal closure, while anisohydric wheat genotypes may have deep root systems that might help to maintain stomatal conductance, by having access to water in deeper soil layers. However, as this study did not include root traits, their roles remain unclear. Additionally, higher planting density might influence this result. Stomatal closure during the reproductive stage improves grain set in wheat, and genetic variations influencing stomatal conductance are presumed to affect a plant's ability to adapt to limited water conditions (Onyemaobi et al., 2021).

We found strong correlations between phenological traits (DTH and DTF) and BM and GY, consistent with those reported by Gizaw et al. (2016), indicating positive correlations between phenological days and BM. Positive associations between phenological traits and GY under rainfed conditions support the importance of crop phenology in a water stress environment in line with previous findings (Lopes et al., 2012). For instance, a diploid landrace of *T. monococcum*, which has a prolonged vegetative period, exhibited advantages of BM accumulation and GY maintenance under water stress, which could ultimately contribute to increased photosynthesis and a decreased reduction in gsw. Our results revealed that individual genotypes within the *Triticum* gene pool under investigation exhibited diverse trait combinations rather than consistent combinations of all the positive traits in a single genotype, indicating variability in drought tolerance (Figs. 2, 3, 4, S1 and S2). Such genotypic variations in drought adaptation among different genotypes and species have previously been studied in wheat (Aberkane et al., 2021; Lopes et al., 2014; Suneja et al., 2017).

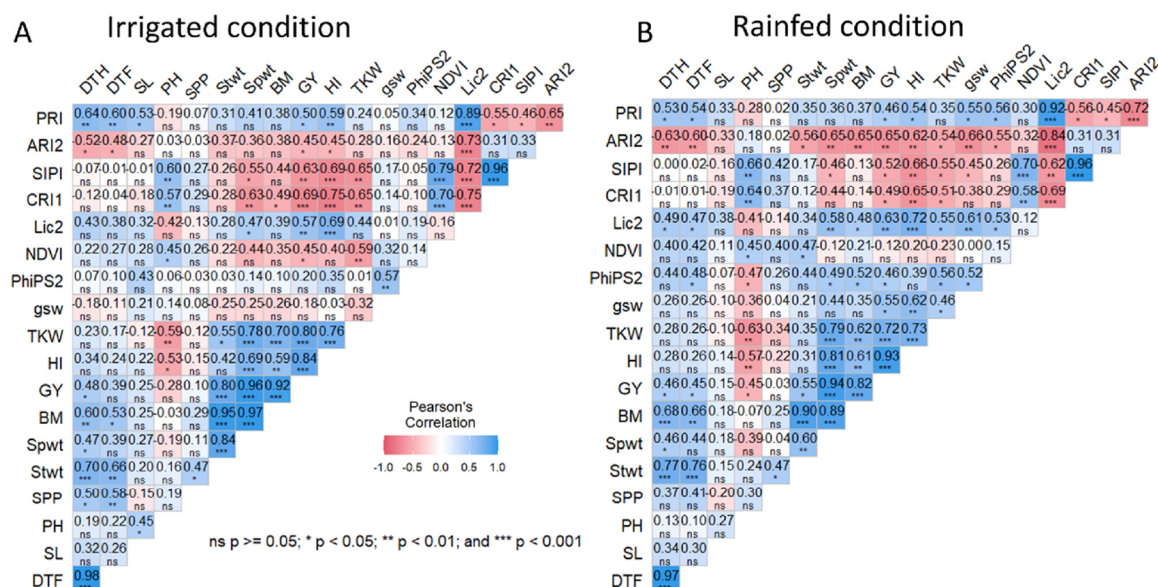


Fig. 5. Pearson's correlation between the 19 measured traits for irrigated (A) and rainfed conditions (B). DTH = days to heading, DTF = days to flowering, PH = plant height, SL = spike length, SPP = number of spikes per plant, Spwt = spike weight per plant, Stwt = straw weight per plant, BM = above ground biomass per plant, GY = grain yield per plant, TKW = thousand kernel weight, HI = harvest index, gsw = stomatal conductance, PhiPS2 = photosystem II efficiency, NDVI = normalized difference vegetation index, Lic2 = Lichtenthaler index 2, SIPI = structure insensitive pigment index, CRI1 = carotenoid reflectance index1, PRI = photochemical reflectance index, and ARI2 = anthocyanin reflectance indices.

4.5. Uncertain genotypic effects on photo-spectral indices under drought stress

Photosynthetic pigment indices reflect physiological changes in plants (Sims and Gamon, 2002). As a measure of chlorophyll content and its spectrum, the NDVI is used to predict green biomass (Liu et al., 2019). Chlorophyll absorbs light, which is integral for the photosynthetic process. Previous studies (Lopes et al., 2014; Raun et al., 2001) demonstrated that NDVI measurements of winter wheat at the reproductive or late flowering stages do not provide reliable predictions of GY and biomass production. These findings support our results, indicating an absence of associations between the NDVI and GY under water stress conditions (Fig. 5B). In contrast, Zhang et al., (2019) revealed that NDVI measured at the flowering and the mid-grain filling stages was positively correlated with yield in wheat under both irrigated and water stress conditions. Therefore, further study is needed considering alternative growth stages of the vegetation indices associated with the chlorophyll index. This can provide a more accurate representation of the relationship between the NDVI and GY under different environmental conditions (Lopes et al., 2014).

Our finding that drought stress does not significantly impact PhiPS2 endorses that of Sommer et al. (2023), who reported that drought stress does not affect PhiPS2 except when the stress is severe or when it is mild, which prompts the activation of several protective mechanisms. Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress (Merzlyak et al., 1999; Sims and Gamon, 2002). The lack of a significant effect of the chlorophyll-to-carotenoid ratio (Lic2) on the plants in our study was likely due to stress conditions during the reproductive stage. However, a complex scenario emerged with a genotype-by-treatment interaction effect on PhiPS2, Lic2, and NDVI, indicating the diverse responses of individual genotypes. Consequently, traits such as PhiPS2, Lic2, and NDVI may lack consistency and may not therefore be reliable criteria for selecting drought-tolerant genotypes under drought stress. Further investigations are required to explore

tolerance mechanisms in specific genotypes, considering diverse growth stages and dynamic drought scenarios to elucidate how these physiological traits reflect drought tolerance in different genotypes.

4.6. Genotype-specific drought adaptation in the Triticum gene pool

Our third hypothesis proposed that drought tolerance is greater in wild wheats than in modern wheat cultivars. To test this hypothesis, we undertook a comprehensive comparison of the drought response of wild wheat genotypes with those of modern cultivars. We found that drought tolerance in the *Triticum* gene pool is genotype specific, which challenges our assumption of the uniform superiority of wheat wild relatives over modern cultivars. This finding emerged during the screening experiment in 2022 (Table S2 and S3) and through the performance of a two-season experiment evaluating the drought response of various genotypes via their morphological and physiological traits (Figs. 2, 3, and 4). This might be because the breeding efforts for high yield may also incorporate useful traits for abiotic and biotic stress tolerance (Mohammadi, 2018). However, in our two-season experiment, *T. urartu* (G45) exhibited greater tolerance than *T. aestivum* cv. Apostel (G134) and cv. Nordkap (G181), and excluding *T. durum* cv. Sambadur. These findings are aligned with those of a previous study (Pour-Aboughadareh et al., 2017), which reported that *T. urartu* is drought-tolerant. Additionally, one study showed that introgression lines derived from *T. urartu* produced greater yields under drought stress than their parental lines (Aberkane et al., 2021). *T. boeoticum* has also been reported to be drought tolerant (Liu et al., 2015; Mehrabad Pour-Benab et al., 2019). Notably, these diploid wild species exhibited greater drought tolerance than bread wheat genotypes.

Our results indicated that *T. dicoccoides* (G90, G242, G248) exhibited superior drought tolerance relating to GY and other yield-related traits, such as BM, Spwt, Stwt, and HI, compared with *T. aestivum* cv. Apostel (G134) and cv. Nordkap (G181) and excluding *T. durum* cv. Sambadur (Fig. 2B). These findings are aligned with those of previous studies

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(Peleg et al., 2005, 2009; Suneja et al., 2017; Zhang and Kirkham, 1994), which identified *T. dicoccoides* as being drought tolerant. *T. dicoccoides* has evolved rich genetic diversity and developed adaptations to adverse environmental stresses, including limited water availability (Budak et al., 2013; Peleg et al., 2005). Our findings support the suggestion of earlier studies that *T. dicoccoides* could potentially be a donor for improving drought tolerance (Aberkane et al., 2021; Merchuk-Ovnat et al., 2016).

In our two-season experiment, 45 % of the wild genotypes belonged to *T. araraticum* and exhibited greater tolerance than modern bread wheat cultivars but less tolerance than *T. durum* cv. Sambadur. This finding contrasts with that of a previous study (Sultan et al., 2012), which reported that *T. araraticum* is less tolerant than bread wheat at an early vegetative growth stage. According to our results, compared with other wild wheat species, *T. araraticum* was less tolerant than *T. dicoccoides*, *T. urartu*, and *T. boeoticum*. However, tolerance varied between the genotypes. We found that *T. araraticum* (G221) was more tolerant than *T. urartu* (G45) and *T. boeoticum* (G27 and G33) but less tolerant than *T. dicoccoides* (G242). The collection sites of *T. araraticum* and *T. dicoccoides* genotypes were, respectively, Iraq and Israel, whereas *T. urartu* and *T. boeoticum* genotypes were respectively collected from Syria and Turkey. Therefore, variations in drought tolerance within wild taxa can be explained by the diverse environments of their regions of origin, which are primarily characterized by a semi-arid to arid climate with hot and dry summers (Jaradat, 2017). Northern Iraq is the center of diversity and the origin of *T. araraticum* (Badaeva et al., 2022). This may suggest that genotypes of *T. araraticum* collected from these areas might have greater drought tolerance, which needs further testing. The *T. araraticum* taxa could augment the diversity of drought tolerance traits in the *Triticum* gene pool along with other wild taxa. They are potentially of considerable value for improving bread and durum wheat (Badaeva et al., 2022). Although, Ayed, et al. (2021) reported that *T. araraticum*-derived lines did not outperform their parent under terminal drought stress, our results suggest that the *T. araraticum* taxa merit further attention as potential candidates for imparting drought tolerance. However, elucidating drought tolerance mechanisms remains challenging, and additional studies are warranted to explore these mechanisms.

5. Conclusion

This study revealed significant variation in morphological and physiological traits within the *Triticum* gene pool in response to water deficit. For example, significant genotype-by-treatment interactions occurred for gsw and GY per plant, as well as vegetation indices. The differential response in terms of gsw and their link to drought-tolerance warrants further investigation and confirmation in plants grown in a community rather than single plants. This study identified some genotypes as potential donors of drought adaptive traits from the wild wheat group, such as *T. dicoccoides* (G242), *T. urartu* (G45), *T. boeoticum* (G27), and *T. araraticum* (G221) in terms of GY per plant with a high response to gsw. Moreover, a landrace, *T. monococcum* sinkajae (G89), and the modern durum wheat cultivar, *T. durum* cv. Sambadur (G41) exhibited low GY loss per plant with a low response in terms of gsw. While wild species were not found to be superior, *per se*, to domesticated ones in terms of drought adaptation, the selected genotypes could potentially be used as donors of traits that could contribute to drought tolerance in domesticated wheat. We recommend further studies exploring the transfer of such traits to domesticated wheat.

Funding information

This work was supported by the German Academic Exchange Service (DAAD) under the project, Programm des Projektbezogenen Personenaustauschs (PPP) [project number 57627943] and the Biodiversity for Opportunities, Livelihoods and Development (BOLD) Project funded by

the Government of Norway [grant number QZA-20/0154]. SP received a PhD scholarship from DAAD. The funders did not have any role in the study design or implementation.

CRedit authorship contribution statement

Sumitra Pantha: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Hakan Ozkan:** Writing – review & editing, Resources. **Benjamin Kilian:** Writing – review & editing, Validation. **Michael Frei:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Frederike Zeibig:** Writing – review & editing, Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sumitra Pantha reports financial support was provided by German Academic Exchange Service. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Liane Renno, Erika Schick, Lisa Marie Moosmann and Mishel Risilia for their assistance during the field experiments and for their help in threshing, cleaning, and measuring the samples. The authors would also like to thank Mario Tolksdorf and his team at the Gross Gerau field station for their support during the field-work. We would like to thank to Dr. Sawitree Autarmat for supporting the experimental work. Lastly, we would also like to thank Radhika Johari for helping in editing the manuscript.

Code availability

Not applicable

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fcr.2024.109678](https://doi.org/10.1016/j.fcr.2024.109678).

Data availability

Data will be made available on request.

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3 Physiological and biochemical changes induced by drought stress during the stem elongation and anthesis stages in the *Triticum* genus

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Published in: *Environmental and Experimental Botany*, 228 (2024) 106047

DOI: 10.1016/j.envexpbot.2024.106047



Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot



Research paper

Physiological and biochemical changes induced by drought stress during the stem elongation and anthesis stages in the *Triticum* genus



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ARTICLE INFO

Keywords:

drought tolerance
drought escape
drought avoidance
Triticum genus
stem elongation
anthesis

ABSTRACT

Drought stress negatively influences the growth, development, and grain yield of wheat by disrupting its morphological, physiological, and biochemical processes. This study examined the effects of drought stress during the stem elongation and anthesis developmental stages of species within the *Triticum* genus along with their drought adaptation mechanisms under fully watered and drought conditions. We tested the following two hypotheses: (1) drought tolerance mechanisms for osmotic and stomatal regulation that lead to oxidative stress are correlated between the stem elongation and anthesis stages and affect grain yield loss, and (2) compared with modern cultivars, wild wheat cultivars exhibit greater drought tolerance. To test these hypotheses, a greenhouse pot experiment was conducted using 17 genotypes of wild wheat relatives and landraces, with modern cultivars included for comparison. Drought stress was induced during the stem elongation and anthesis stages until the average soil moisture was approximately 15 % and 18 %, respectively, of the pot's water holding capacity. The soil moisture was maintained at 80–90 % for the fully watered treatment. An examination of physiological and biochemical traits revealed that drought significantly reduced stomatal conductance (gsw) and relative water content (RWC) during both developmental stages. However, significant increases occurred in the malondialdehyde (MDA) content during both stages and in the proline content during the anthesis stage. Drought stress significantly decreased the number of days to heading and anthesis, indicating that drought escape occurs under severe drought stress. Furthermore, drought significantly decreased morphological and yield-related traits, with the greatest reduction (51 %) occurring in grain yield. Weakly significant positive associations of biochemical and some physiological traits between the stem elongation and anthesis stages partially confirmed our first hypothesis, whereas our results relating to the second hypothesis were inconclusive. We observed genotype-dependent responses to drought stress during both stages for various measured traits. No associations of RWC, proline, or MDA with grain yield were found. However, stomatal conductance was negatively correlated with grain yield under drought stress at the anthesis stage. Certain wild wheat genotypes and landraces exhibited drought avoidance, escape, and tolerance mechanisms, which positively contributed to grain yield. We identified *T. monococcum* subsp. *sinskajae*, *T. boeoticum* and *T. dicoccoides* as the most drought-tolerant genotypes. The findings of this study provide important insight for understanding the drought adaptation traits and their use in wheat breeding programs.

Abbreviations: ARI2, anthocyanin reflectance index 2; BM, aboveground biomass; CRI2, carotenoid reflectance index 2; DTA, days to anthesis; DTH, days to heading; DTM, days to maturity; ET, effective tillers; GN, grain numbers; Gsw, stomatal conductance; GY, grain yield; HI, harvest index; Lic2, Lichtenthaler index 2; MCARI1, modified chlorophyll absorption reflectance index 1; MDA, malondialdehyde; NDVI, normalized difference vegetation index; PCA, principal component analysis; PEL, peduncle exertion length; PH, plant height; PhiPS2, photosystem II efficiency; PWHC, pot soil water-holding capacity; RWC, relative water content; SL, spike length; SHL, shoot length; TT, total tillers; TKW, thousand kernel weight.

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<https://doi.org/10.1016/j.envexpbot.2024.106047>

Received 12 July 2024; Received in revised form 7 November 2024; Accepted 18 November 2024

Available online 19 November 2024

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1. Introduction

The frequency and severity of drought stress have increased due to climate change, which poses a serious threat to crop production globally (Munné-Bosch and Villadangos, 2023; Shehzad et al., 2023; Vicente-Serrano et al., 2022). Drought stress induces morphological, physiological, biochemical, and molecular changes in plants (Sallam et al., 2019; Shelake et al., 2022). These changes affect plant growth and development, substantially reducing crop yields (Farooq et al., 2014; Shelake et al., 2022). The severity of drought stress depends on its intensity and frequency, as well as on the crop's phenological stage (Chaouachi et al., 2023; Wang et al., 2022). The anthesis and grain-filling developmental stages in wheat are most sensitive to drought stress, which affects grain yield (Sarto et al., 2017; Shokat et al., 2023). However, wheat plants can be affected by drought stress at any growth stage, including seedling, tillering, and stem elongation (Sallam et al., 2019; Wang et al., 2015). When plants are exposed repetitively to drought stress, they may develop drought stress memory, which enables them to respond faster to further water stress, thus providing greater protection (Jacques et al., 2021; Wang et al., 2014, 2015). However, some biochemical and physiological changes caused by repetitive and severe droughts could be ineffective in providing protection (Krasensky and Jonak, 2012). In addition, severe drought stress events at the stem elongation stage affect leaf initiation and expansion, reducing plant biomass and the number of grains per spike and ultimately limiting grain yield (Blum et al., 1990; Saeidi et al., 2015).

The negative impacts of drought stress caused by cell dehydration include restricted cell elongation, stomatal closure and a reduction in photosynthetic activity, thereby inhibiting plant growth and development (McAusland et al., 2020; Chaouachi et al., 2023; Tambussi et al., 2002). The initial response of plants to drought stress is stomatal closure, which is beneficial for their survival under drought, as it reduces water loss from leaves (Nolan et al., 2017). However, this causes a decrease in the CO₂ assimilation rate, so plants can absorb more light than they actually use for photosynthesis (Li et al., 2017). Consequently, drought stress triggers the production of reactive oxygen species resulting from the impairment of photochemical reactions in the chloroplast, which leads to oxidative stress in the plant and damage to cell membranes due to lipid peroxidation (Abid et al., 2018; Hayat et al., 2012).

The drought stress response in plants is a complex phenomenon that involves changes in plant morphology and physiology and in biochemical pathways (Bhatta et al., 2018; Shelake et al., 2022). This process depends on a range of adaptive mechanisms, which include escape from drought as well as drought avoidance and tolerance (Bapela et al., 2022; Farooq et al., 2014; Sallam et al., 2019). The escape strategy involves accelerated crop phenology or a shortening of the plants' growth period to complete their life cycle (Shavrukov et al., 2017). Drought avoidance is a water-saving strategy involving different morphophysiological changes, such as a reduction in stomatal conductance by lowering water use or preventing dehydration (Bandurska, 2022; Martínez-Vilalta and García-Fórner, 2017). Plants also exhibit an avoidance strategy characterized by reduced plant growth and development, which results in decreases in plant height, tiller number, and biomass (Anyia and Herzog, 2004; Izanloo et al., 2008; Zia et al., 2021).

A drought tolerance strategy involves the regulation of physiological and biochemical changes in plants (Farooq et al., 2014; Sallam et al., 2019). This process mainly hinges on osmotic adjustments to maintain cell volume and turgor pressure, enabling plants to continue growing under drought stress (Izanloo et al., 2008; Nolan et al., 2017). Osmotic adjustment lowers the osmotic potential and maintains the water status of plants, thereby maintaining their cellular functions during osmotic stress (Abid et al., 2018; Ayed et al., 2021). Osmoprotectants, such as proline, can be triggered by drought stress to help detoxify oxidative stress and maintain osmotic adjustments (Shrestha et al., 2022). Plants may utilize either individual mechanisms or a combination of the above mechanisms in response to drought stress.

Common or bread wheat (*Triticum aestivum* L., genome BBAADD, 2n = 6x = 42) is a major cereal crop worldwide. It is a key source of calories and proteins for the global population (Food and Agricultural Organization FAO, 2023; Langridge et al., 2022). Wheat wild relatives and landraces may have developed specific adaptations to their local environments and therefore offer valuable genetic resources for introducing drought tolerance into breeding programs (Aberkane et al., 2020; Ahmadi et al., 2018; Leigh et al., 2022; Lopes et al., 2015; Nevo and Chen, 2010; Peng et al., 2011). Drought stress affects the morphological, physiological, and biochemical traits of wheat plants (Ayed et al., 2021; Pour-Aboughadareh et al., 2017; Suneja et al., 2017). Previous studies have examined drought adaptation traits in different wheat genotypes, which vary from the field to the gene level (Farrell et al., 2017; Lopes et al., 2014; Ma et al., 2017; Nevo and Chen, 2010; Reynolds et al., 2007; Wen et al., 2023). Stress tolerance mechanisms such as stomatal regulation, maintenance of water content, and proline production have mostly been studied in domesticated wheat (Ahmadi et al., 2018; Mwadzingeni, et al., 2016; Saghour el idrissi et al., 2023). Additionally, some wild species, such as *T. dicoccoides*, *T. boeoticum*, and *T. urartu*, have been investigated for their drought stress physiology (Peleg et al., 2009; Pour-Aboughadareh et al., 2017; Suneja et al., 2017). However, linkages between their drought adaptation mechanisms and yield components have not been fully explored. Furthermore, despite its significant potential for wheat breeding, *T. araraticum* (GGA¹A¹), a wild tetraploid, has not been widely studied (Badaeva et al., 2022). To the best of our knowledge, very few studies have addressed the abiotic stress physiology of *T. araraticum* (GGA¹A¹) (Badaeva et al., 2022; Sultan et al., 2012). Moreover, few studies have addressed the effects of drought stress caused by repetitive drought events during the stages of stem elongation and anthesis in wild wheats and landraces (Bapela et al., 2022; Nevo and Chen, 2010). Therefore, an understanding of stress-adaptive changes during these two stages and the associated mechanisms to maintain stable yield under drought stress is crucial for advancing wheat breeding programs.

The effects of drought stress on a diverse panel of *Triticum* taxa have not been fully characterized, and the underlying mechanisms of drought stress tolerance have yet to be elucidated. It is therefore necessary to explore a wide range of genetic diversity to gain a better understanding of individual drought tolerance mechanisms (Bao et al., 2023; Lopes et al., 2014, 2015). Therefore, the objective of this study was to investigate the effects of drought stress on morphophysiological traits associated with biochemical markers in different genotypes of the *Triticum* genus. Accordingly, we conducted a pot experiment under greenhouse conditions with drought simulations introduced during the stem elongation and anthesis developmental stages under two different treatments: well-watered and drought stress conditions. We used a diverse panel of wild wheat, landraces, and modern cultivars belonging to three different ploidy levels and eight different taxa. The following hypotheses were formulated:

- (1) Drought tolerance mechanisms for osmotic and stomatal regulation counteracting oxidative stress are correlated between the stem elongation and anthesis stages and affect grain yield loss;
- (2) Compared with modern cultivars, wild wheat plants exhibit greater drought tolerance.

An experiment to verify these hypotheses can provide valuable input for developing drought-tolerant cultivars in wheat breeding programs by incorporating different drought tolerance mechanisms.

2. Materials and methods

2.1. Plant material

A total of seventeen genotypes were used in this experiment, comprising eleven wild wheat samples from four taxa, three

domesticated landraces from three taxa, and three modern cultivars from two taxa (Table 1). The wheat taxa were categorized according to the classification system outlined in a previous study (Zeibig et al., 2024). The accessions were selected on the basis of the results of previous field experiments. The wild wheat genotypes and domesticated landraces were sourced from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany, and from Çukurova University, Turkey. For comparison purposes, two modern bread wheat cultivars, *T. aestivum* cv. Apostel (I.G. Pflanzenzucht GmbH) and cv. Nordkap (SAATEN UNION), and one modern durum wheat cultivar, *T. durum* cv. Sambadur (Hauptsaaften für die Rheinprovinz GmbH), were included in the study.

Table 1
Classification of genotypes into taxa, biological status, and ploidy levels, with their countries of origin.

Taxon	Biological status	Genome	Genotype	Country of origin and seed collection site
Diploid, 2n = 2x = 14				
<i>T. urartu</i> Thumanjan ex Gandilyan.	Wild	A ^a	G45	Syria: 3 km northwest of Salah; on the road to Sousan
<i>T. boeoticum</i> Boiss.	Wild	A ^b	G27	Turkey: 52.5 km northeast of Urfa, near Hilvan
<i>T. boeoticum</i> Boiss.	Wild	A ^b	G33	Turkey: 69 km northwest of Kirsehir
<i>T. monococcum</i> subsp. <i>sinskajae</i> (Filat. & Kurkiew)	Landrace	A ^o	G89	Turkey
Tetraploid, 2n = 4x = 28				
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G90	Turkey: 9.3 km southeast from Ergani toward Diyarbakir
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G242	Israel: Tabigha
<i>T. dicoccoides</i> (Körn. ex Asch. & Graebn.) Schweinf.	Wild	BA	G248	Iraq: northern slope of Jabal Sinjar north of Kursi
<i>T. durum</i> Desf.	Modern cultivar	BA	G41	cv. Sambadur (Hauptsaaften), Germany
<i>T. araraticum</i> Jakubz.	Wild	GA ^f	G161	Iraq: 10.6 km east-northeast from Koi Sanjaq toward Ranya
<i>T. araraticum</i> Jakubz.	Wild	GA ^f	G189	Iran
<i>T. araraticum</i> Jakubz.	Wild	GA ^f	G205	Iraq: 33.2 km west from Rowanduz toward Shaqlawa
<i>T. araraticum</i> Jakubz.	Wild	GA ^f	G221	Iraq: 4.4 km northwest from Amadiyah, Mazorka Gorge
<i>T. araraticum</i> Jakubz.	Wild	GA ^f	G222	Iraq: 19.3 km south from Sulaymaniyah toward Qara Dagh
Hexaploid, 2n = 6x = 42				
<i>T. aestivum</i> L.	Landrace	BAD	G10	Afghanistan: Herat and Palpiri
<i>T. aestivum</i> L.	Modern cultivar	BAD	G134	cv. Apostel (I.G. Pflanzenzucht), Germany
<i>T. aestivum</i> L.	Modern cultivar	BAD	G181	cv. Nordkap (SAATEN UNION), Germany
<i>T. spelta</i> L.	Landrace	BAD	G109	Iran: Noach, 18 km from Shahr-Kord

2.2. Experimental conditions and treatments

The experiment was conducted during the 2022 winter wheat growing season (from October 2022 to May 2023) at Justus-Liebig-University, Germany, under controlled greenhouse conditions. A minimum photosynthetically active photon flux density (PPFD) of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the light period was ensured by natural and artificial light in the greenhouse. Eighteen uniformly sized seeds of each genotype were sown in Quickpot trays on October 4, 2022. After 48 hours of stratification at 5 °C, the seedlings were germinated in a greenhouse under light/dark conditions of 10 h/14 h and day/night temperatures of 15 °C/10 °C. After germination, the plants continued to grow until they reached the 2–3 leaf stage (Z12; Zadoks et al., 1974) under light/dark conditions of 8 h/16 h and day/night temperatures of 10 °C/5 °C and a relative humidity of .55–60 %. The plants underwent vernalization under natural conditions inside a netted chamber for two months and were subsequently allowed to harden under greenhouse conditions (light/dark conditions of 8 h/16 h and day/night temperatures of 10 °C/5 °C) for two weeks. The plants were then transplanted to 5 L plastic pots filled with N-type soil substrate (Hawita Gruppe GmbH, Germany) for six replications in total, with two plants per pot in three replications and one plant per pot in the remaining three replications. A randomized complete block design was used with two treatments: well-watered conditions and drought stress conditions. After one week, the following growth conditions were used: a light/dark period of 10 h/14 h and day/night temperatures of 15 °C/10 °C to promote gradual acclimatization over one week, followed by a light/dark period of 16 h/8 h, day/night temperatures of 21 °C/16 °C, and 55–60 % relative humidity maintained until crop maturity.

The soil moisture content was measured with an HH2 soil moisture meter (Delta-T Devices Ltd., version 4.2, Cambridge, UK), and the volumetric water content was assessed to provide a reference for the pot soil water-holding capacity (PWHC) in a 5 L pot. Measurements of moisture content were taken every two to three days from the time of transplanting until the plants reached physiological maturity (Figure S1). Well-watered plants received regular irrigation to maintain the soil moisture content at 80–90 % of the PWHC throughout the growth period. Drought stress treatment during the stem elongation stage commenced at Z25 (Zadoks et al., 1974), with cessation of irrigation for 16 days until the average soil moisture reached a level below 15 % of the PWHC. After the physiological parameters were measured and a fresh sample was harvested during the stem elongation stage, 16 days after the drought stress treatment, the plants were rewatered to restore the soil moisture to 80–90 % of the PWHC, which was consistent with that of the well-watered treatment group. A second simulation was implemented targeting the anthesis stage, specifically for individual genotypes at Z49, to induce drought stress during the anthesis stage (Z60 to Z73; Zadoks et al., 1974) until the average soil moisture decreased to 18 % of the PWHC. The same potted plants were subjected to drought stress at both the stem elongation and anthesis stages. Following the collection of physiological data and the harvest of fresh samples during the anthesis stage, the plants were rewatered to maintain the soil moisture at 80–90 % PWHC until physiological maturity was attained (Z93).

2.3. Harvest of fresh samples

The plants were harvested fresh during the stem elongation stage after 16 days of drought stress treatment. For all the genotypes, one plant from each of the first three replications that contained two plants per pot was harvested from both the well-watered and drought treatments and flash frozen in liquid nitrogen. Fresh harvesting during drought treatment at the anthesis stage was performed at Z73 (Zadoks et al., 1974) for individual genotypes when the average soil moisture decreased to 18 % of the PWHC. Two to three leaves below the flag leaf were harvested at the anthesis stage for biochemical analysis.

Immediately after the fresh harvest, all samples from both the stem elongation and anthesis stages were stored at -80°C for biochemical analysis. All the study parameters were measured during drought episodes for both the well-watered and drought stress treatments.

2.4. Agro-morphological traits

During the stem elongation stage, shoot length (SHL) was measured from the soil surface to the tip of the longest, fully expanded leaf, and total tillers (TT) were counted 16 days after the drought stress treatment prior to harvesting the fresh samples. Phenological data, such as days to heading (DTH) and days to anthesis (DTA), were recorded for individual plants at Z50 and Z60 (Zadoks et al., 1974), respectively. Days to maturity (DTM) were recorded when the main spike turned fully yellow at Z90. The phenological days were recorded with the seed sowing date to provide reference values for the respective phenological stages. Plant height (PH) was measured from the soil surface to the top of the spike (excluding the awns). Spike length (SL) was measured from the base to the top of the spike (excluding the awns). The peduncle exertion length (PEL) was measured from the base of the flag leaf sheath to the base of the spike. PH, SL, and PEL were measured after anthesis. The number of effective tillers (ET) was counted after the plants reached physiological maturity. The aboveground biomass weight (BM) per plant was measured after the sample was oven-dried at 30°C to reach a constant weight. The spikes were then threshed in a coffee mill, after which the grain yield (GY) per plant and thousand kernel weight (TKW) were measured. For each plant, 50 seeds were counted three times, and the TKW was calculated with reference to the average weight of 50 seeds. The grains were measured for TKW, and the number of grains (GN) per plant was determined via a Contador Seed Counter (Pfeuffer GmbH, Kitzingen, Germany). The harvest index (HI) for each plant was calculated as a percentage by dividing the GY by the BM per plant.

2.5. Physiological traits

Spectral reflectance measurements (vegetation indices) were recorded using a Polyphen RP 410 portable instrument (Photon Systems Instruments, Drasov, Czech Republic). The following indices were calculated: normalized difference vegetation index (NDVI) = $(R780 - R630)/(R780 + R630)$; Lichtenthaler index 2 (Lic2) = $R440/R690$ (Begum et al., 2020); anthocyanin reflectance indices (ARI2) = $R790/(1/R550 - 1/R700)$ (Gitelson and Merzlyak, 1997); carotenoid reflectance index 2 (CRI2) = $1/R510 - 1/R700$ (Gitelson et al., 2003); and modified chlorophyll absorption reflectance index (MCARI1) = $1.2 \times [2.5 \times (R790 - R670) - 1.3 \times (R790 - R550)]$ (Daughtry et al., 2000). A portable handheld LI-600 porometer system integrated with a fluorometer (LI-COR, Lincoln, Nebraska, USA) was used to measure stomatal conductance (gsw) and photosystem II efficiency (PhiPS2). All of these measurements were performed before the harvest of fresh samples during both the stem elongation and anthesis stages under drought stress.

The water status of the leaves was measured by the relative water content (RWC). To measure RWC, a mid-leaf section (approximately 4–5 cm) was cut from the leaf, and the fresh weight (FW) was immediately recorded. The leaf section was subsequently dipped in a 15 ml Falcon tube filled with 10 ml of deionized water for 24 h at room temperature. The leaf sample was removed from the tube, and the turgid weight (TW) was measured immediately after excess water was removed with tissue paper. Dry weight (DW) was recorded after the leaf sections were oven-dried at 80°C for 48 h. RWC was calculated as $\text{RWC} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100$.

2.6. Biochemical traits

The proline content was analyzed applying the method developed by Bates et al. (1973), with slight modifications. Sulfosalicylic acid (3 %

w/v) and ninhydrin reagents (2.5 g of ninhydrin in 60 ml of glacial acetic acid and 40 ml of 6 M phosphoric acid) were used to determine the proline content. In brief, the samples were ground in liquid nitrogen, and 50 mg of the chilled sample was placed in a 2 ml microcentrifuge tube. Next, 1.5 ml of 3 % sulfosalicylic acid was added, and the mixture was vortexed vigorously before being centrifuged at $12,000 \times g$ for 10 min. Then, 500 μl of the supernatant was added to a 10 ml glass test tube, 500 μl of acetic acid and 500 μl of ninhydrin reagents were added, and the mixture was vortexed thoroughly. The mixture was subsequently incubated at 100°C for one hour. The reaction was immediately stopped by placing the tube on ice, and 1.5 ml of pure toluene was added, vortexed, and left at room temperature for 30 min. The absorbance of the chromophore was measured at 520 nm via a microplate reader (Infinite 200 Pro, Tecan, Groedig, Austria). The sample proline content was calculated using the standard curve approach and expressed in micrograms per gram of fresh weight ($\mu\text{g g}^{-1}\text{FW}$).

Lipid peroxidation was determined according to the malondialdehyde (MDA) concentration using the thiobarbituric acid (TBA) method described by Ali et al. (2019). In brief, 100 mg of fresh tissue was homogenized in 1.5 ml of 0.1 % (w/v) trichloroacetic acid (TCA) and centrifuged at $14,000 \times g$ for 15 min at 4°C . The reaction mixture (RSI) was prepared by dissolving 0.01 % (v/v) 2,6-di-tert-butyl-4-methylphenol (BHT) in 20 % TCA (v/v), and the reaction mixture (RSII) was prepared by dissolving 0.65 % TBA (w/v) in warm RSI solution. Next, 500 μl of plant extract was added to 500 μl of RSI and 500 μl of RSII in separate 15 ml Falcon tubes. The reaction mixture was incubated at 95°C for 30 min. After incubation, the reaction was stopped by placing the tube on ice for 5 min, and the reaction mixture was then transferred to a 1.5 ml centrifuge tube and centrifuged at $8000 \times g$ for 10 min at 4°C . The absorbance was measured at 440, 532, and 600 nm using a microplate reader (Infinite 200 Pro, Tecan, Groedig, Austria).

2.7. Data analysis

To rank the genotypes for drought tolerance, a relative value was calculated for each genotype using the method described by Bouslama and Schapaugh (1984), with slight modifications. Specifically, it was calculated for each genotype as the ratio of the drought value divided by the average well-watered value for each studied trait. Relative values are useful for comparing highly variable genotypes across different taxa. The genotypes were sorted and arranged in ascending order, from low to high relative values in the bar graphs. The statistical analysis was performed in R Studio with R version 4.2.0 (www.r-project.org). Analysis of variance (ANOVA) was performed via a mixed linear model in the nlme package (<https://cran.r-project.org/web/packages/nlme/index.html>), with genotype and treatment considered fixed factors and replication by treatment interaction considered a random effect. Multiple comparison tests were performed via the emmeans package (<https://cran.r-project.org/web/packages/emmeans/index.html>). The correlation plots were generated via multi-environment trial analysis (<https://cran.r-project.org/web/packages/metan/index.html>), (<https://cran.r-project.org/web/packages/ggpubr/index.html>) and the corrplot (<https://cran.r-project.org/web/packages/corrplot/index.html>) package in R to determine Pearson's correlation. Principal component analysis (PCA) was conducted using FactoMineR (<https://cran.r-project.org/web/packages/FactoMineR/index.html>) and Factoextra (<https://cran.r-project.org/web/packages/factoextra/index.html>). Figures were generated with ggplot2 (<https://cran.r-project.org/web/packages/ggplot2/index.html>) and Microsoft Excel.

3. Results

3.1. Changes in physiological traits

At both the stem elongation and anthesis stages and across all the genotypes, gsw and RWC decreased significantly due to drought stress

Table 2

Descriptive statistics and analysis of variance (ANOVA) for the physiological and biochemical traits of 17 genotypes from the *Triticum* genus measured at the stem elongation and anthesis stages.

Traits	Mean (Standard Deviation)		ANOVA		
	Well-watered	Drought	Treatment	Genotype	Treatment × Genotype
Stem elongation stage					
Relative water content (%)	91.7 (3.3)	61.9 (15.1)	**	*	**
Proline (µg/g, FW)	56.1 (15.5)	3998.6 (1973.9)	**	****	****
Malondialdehyde (nmol/ml/g, FW)	16.4 (5.4)	25.2 (5.4)	**	****	*
gsw (mmol m ⁻² s ⁻¹)	416.2 (155.7)	52.5 (77.1)	****	****	****
PhiPS2	0.64 (0.09)	0.65 (0.08)	ns	**	ns
NDVI	0.60 (0.03)	0.59 (0.04)	ns	****	ns
Lic2	0.97 (0.16)	1.00 (0.17)	ns	**	ns
CRI2	1.48 (0.85)	1.18 (0.80)	ns	**	ns
ARI2	-0.21 (0.17)	-0.25 (0.19)	ns	***	ns
MCARI1	2.01 (2.21)	2.35 (2.44)	ns	*	ns
Anthesis Stage					
Relative water content (%)	89.1 (6.5)	65.0 (15.5)	*	*	*
Proline (µg/g, FW)	53.7 (10.6)	1856.5 (1578.6)	ns	***	***
Malondialdehyde (nmol/ml/g, FW)	10.1 (2.5)	16.1 (3.5)	**	****	ns
gsw (mmol m ⁻² s ⁻¹)	239.6 (244.5)	52.1(96.9)	****	****	****
PhiPS2	0.64 (0.09)	0.66(0.10)	ns	**	*
NDVI	0.57 (0.05)	0.57(0.05)	ns	****	ns
Lic2	0.89 (0.08)	0.91(0.11)	*	****	ns
CRI2	1.71 (0.56)	1.34(0.67)	**	****	ns
ARI2	-0.11 (0.08)	-0.14(0.12)	ns	****	ns
MCARI1	0.69 (0.05)	0.73(0.07)	**	****	**

Note: Mean values across all genotypes are shown. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. ns = nonsignificant, FW = fresh weight, gsw = stomatal conductance, PhiPS2 = photosystem II efficiency, ARI2 = anthocyanin reflectance index 2, CRI2 = carotenoid reflectance index 2, Lic2 = Lichtenthaler index 2, MCARI1 = modified chlorophyll absorption reflectance index 1, NDVI = normalized difference vegetation index.

(Table 2). On average, gsw and RWC decreased by 87 % and 32 %, respectively, during the stem elongation stage and by 78 % and 27 %, respectively, during the anthesis stage. However, the Lic2 and MCARI1 vegetative indices increased significantly across all the genotypes due to drought stress, on average, by 25 % and 5 %, respectively. At the stem elongation stage, CRI2 decreased significantly by 22 %, but no such effect was observed for the vegetation indices (Table 2). All the genotypes showed significant gsw responses to the drought stress treatment during the stem elongation stage (Fig. 1A). However, during the anthesis stage, not all of them revealed significant gsw responses to drought stress (Fig. 1B). Whereas drought stress induced significant reductions in gsw in *T. boeoticum* (G33, G27), *T. urartu* (G45), and *T. dicoccoides* (G242), reductions in gsw were lower in *T. araraticum* (G161 and G189).

Under drought stress, the RWC decreased in all the genotypes during both the stem elongation and anthesis stages. However, the decrease was not significant in some of the genotypes (Figs. 1C and 1D). The lowest decrease in RWC was observed for *T. monococcum* subsp. *sinskajae* (G89), *T. urartu* (G45), *T. araraticum* (G205), and *T. boeoticum* (G33) during the stem elongation stage (Fig. 1C). During the anthesis stage, decreases in RWC were lowest for *T. boeoticum* (G27), *T. spelta* (G109), *T. dicoccoides* (G242), *T. urartu* (G45), and *T. araraticum* (G205) (Fig. 1D). During both stages, these genotypes showed average relative values >0.8. The genotype effect was significant for all physiological traits, such as RWC, gsw, PhiPS2, NDVI, Lic2, ARI2, CRI2, and MCARI1, during both the stem elongation and anthesis stages (Table 2). The treatment × genotype effect was highly significant for RWC and gsw during the stem elongation stage and was significant for various physiological traits, such as RWC, gsw, PhiPS2, and MCARI1, during the anthesis stage (Table 2).

3.2. Changes in malondialdehyde content

Across all the genotypes, the MDA content increased significantly by 53 %, on average, during the stem elongation stage and by 58 % during the anthesis stage due to drought stress (Table 2). The effect of genotype on the MDA content was highly significant during both stages. However, the treatment × genotype interaction effect was significant only during the stem elongation stage. The magnitude of the increase in MDA was lower in *T. aestivum*, cv. Nordkap (G181), *T. aestivum*, cv. Apostel

(G134), and *T. araraticum* (G205), but it increased significantly in *T. durum*, cv. Sambadur (G41) and *T. araraticum* (G221) during the stem elongation stage (Fig. 2A). Similarly, during the anthesis stage, the magnitude of the increase in MDA was lower in *T. araraticum* (G189, G221, and G222), *T. aestivum*, cv. Nordkap (G181), *T. dicoccoides* (G242), and *T. spelta* (G109), whose relative values were <1.5 in the drought scenario (Fig. 2B). The magnitude of the increase in MDA was significantly greater in *T. boeoticum* (G33), *T. durum*, cv. Sambadur (G41), and *T. dicoccoides* (G90) than in the other genotypes.

3.3. Changes in proline content

During both the stem elongation and anthesis stages, the average proline concentration increased across all the genotypes due to drought stress (Table 2). During the stem elongation stage, proline increased significantly approximately 71-fold, and during the anthesis stage, it increased approximately 35-fold, which was close to significance (*p* = 0.08; not shown). Furthermore, the effects of genotype and the treatment × genotype interaction were highly significant during both stages. During the stem elongation stage (Fig. 2C), most of the genotypes apart from *T. monococcum* subsp. *sinskajae* (G89), *T. araraticum* (G205), *T. urartu* (G45), *T. boeoticum* (G33) and *T. dicoccoides* (G90) presented significant increases in proline content induced by drought stress. In contrast, during the anthesis stage (Fig. 2D), only *T. dicoccoides* (G90) showed a significant increase (approximately 51-fold) in proline content. During the stem elongation stage, *T. araraticum* (G221) revealed the greatest increase in proline content (approximately 125-fold).

3.4. Changes in crop phenology, morphology, and yield-related traits

Drought stress significantly affected the onset of phenological stages, such as DTH and DTA, whose values were averaged across all the genotypes (Table 3). However, not all the genotypes evidenced significant phenological acceleration. Significant DTA enhancement only occurred in some genotypes, such as *T. urartu* (G45), *T. dicoccoides* (G242), *T. monococcum* subsp. *sinskajae* (G89), *T. boeoticum* (G33), and *T. dicoccoides* (G90) (Figure S2A), whereas significant DTM enhancement was observed only in *T. dicoccoides* (G242 and G248) (Figure S2B).

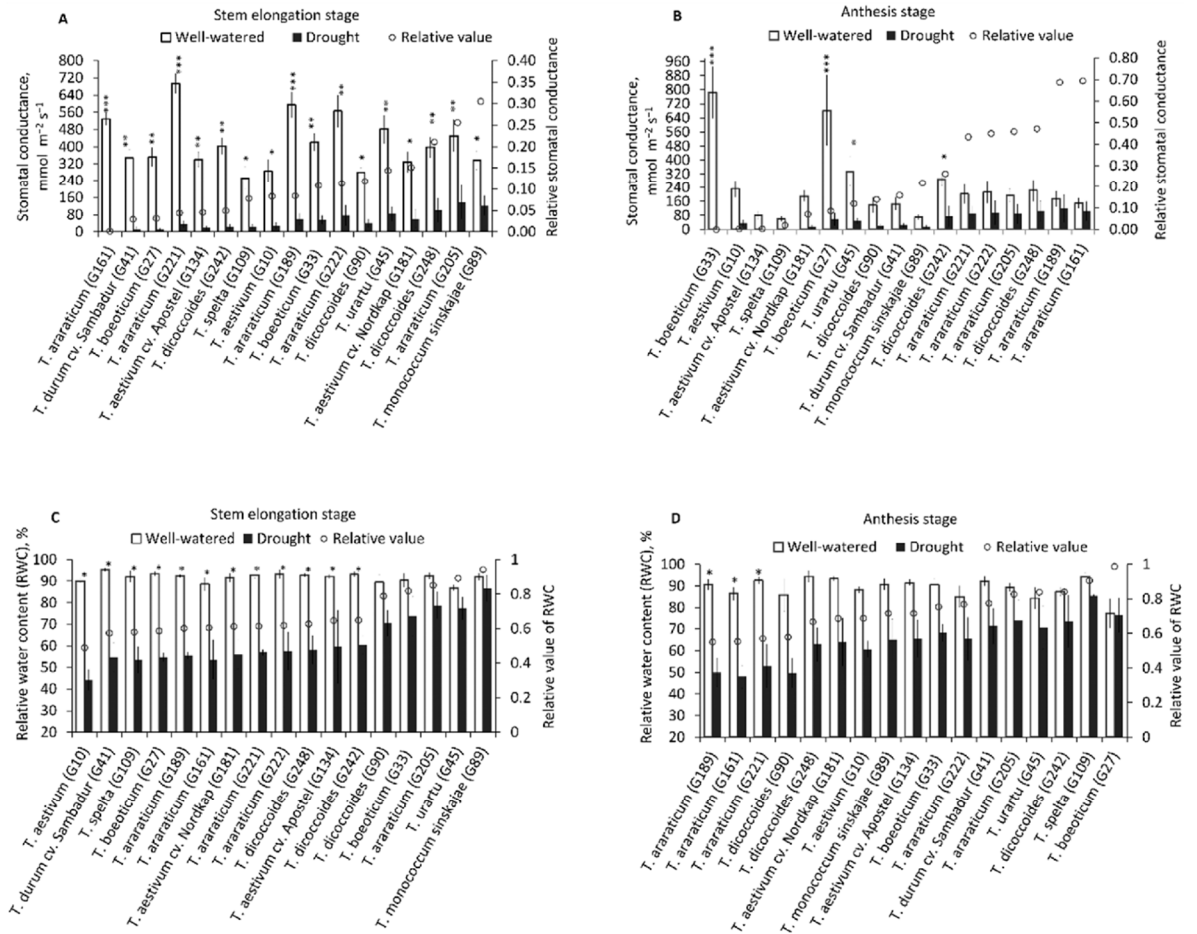


Fig. 1. Stomatal conductance at the stem elongation stage (A) and the anthesis stage (B) and relative water content at the stem elongation stage (C) and the anthesis stage (D). The unshaded circles indicate the mean relative values (drought/well-watered) on the secondary vertical axis, and the genotypes are arranged in ascending order from low relative values (left) to high relative values (right). The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are the average values of five replicates \pm standard errors for stomatal conductance at the stem elongation stage, the average values of six replicates \pm standard errors for stomatal conductance at the anthesis stage, and the average values of three replicates \pm standard errors for relative water content at both stages. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

Drought stress had a severe effect on yield-related traits, such as GY, BM, and GN, which were reduced by 51 %, 49 %, and 46 %, respectively, across all the genotypes (Table 3). However, not all the genotypes exhibited significant decreases in GY, BM, and GN. The decrease in GY was lower for *T. monococcum* subsp. *sinskajae* (G89), *T. boeoticum* (G27), and *T. dicoccoides* (G242), with a relative value >0.6 , and the greatest decrease was observed for *T. aestivum* (G10) (Fig. 3A). *T. monococcum* subsp. *sinskajae* (G89) and *T. aestivum* (G10) exhibited the lowest and greatest decreases in BM, respectively (Fig. 3B). *T. monococcum* subsp. *sinskajae* (G89) showed no GN-related response to drought stress (Fig. 3C). The average TKW and HI values obtained across all the genotypes revealed that their treatment effects were nonsignificant. However, the treatment \times genotype effect was highly significant for both of these traits (Table 3). Drought stress induced significant decreases in TKW in *T. monococcum* subsp. *sinskajae* (G89) and *T. aestivum* (G10). In contrast, some genotypes under drought stress, notably *T. dicoccoides* (G242 and G248), *T. aestivum* cv. Nordkap (G181), *T. boeoticum* (G27 and G33), and *T. urartu* (G45), presented no response to TKW, with relative values of approximately 1. Interestingly, drought

stress induced a significant increase in the HI for *T. dicoccoides* (G242) (Fig. 3E), whereas the HI for *T. aestivum* cv. Apostel (G134) decreased significantly.

The average values across all the genotypes indicated that morphological traits, such as PH, ET, SL, and PEL, were significantly negatively affected by drought stress (Table 3). PH decreased significantly in the genotypes belonging to all four wild taxa and the three landrace taxa but not in the three modern cultivars (Fig. 3F). Genotype effects and treatment \times genotype interaction effects were highly significant for all phenological, morphological, and yield-related traits apart from ET, DTH, and DTA, which were significant for genotype and treatment effects (Table 3). We also investigated drought stress responses related to TT and SHL, which were obtained as average values for all the genotypes during the stem elongation stage. Both of these traits showed significant treatment and genotype effects (Table 3). However, the treatment \times genotype effect was significant only for SHL.

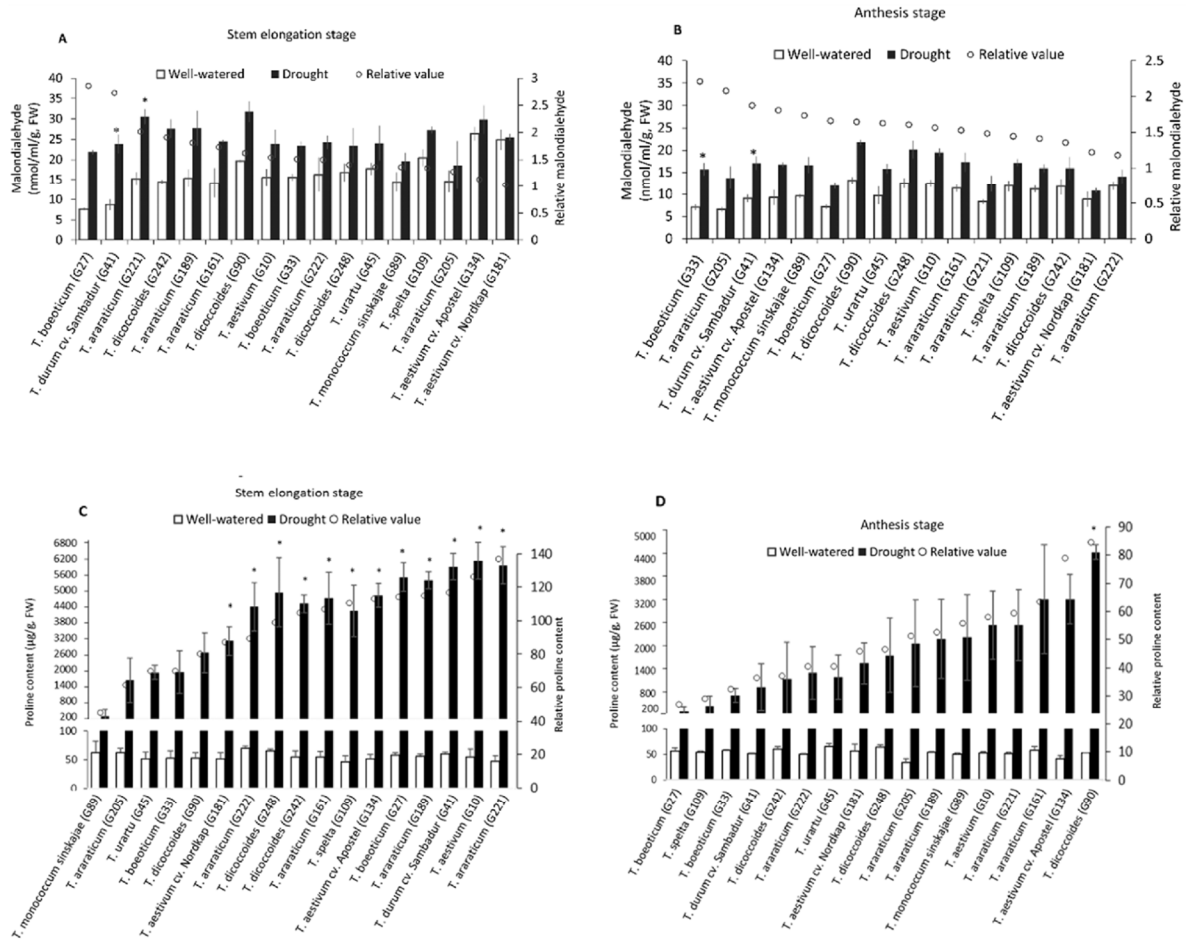


Fig. 2. Malondialdehyde content at the stem elongation stage (A) and the anthesis stage (B); proline content at the stem elongation stage (C) and the anthesis stage (D). The unshaded circles indicate the mean relative values (drought/control) on the secondary vertical axis, and the genotypes are arranged from low relative values (left) to high relative values (right) for proline and from high relative values (left) to low relative values (right) for malondialdehyde. The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are the average values of three replicates \pm standard errors. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.5. Statuswise responses to drought stress for various traits during the stem elongation and anthesis stages

We investigated the responses of the plants to drought stress according to their biological status for morphological, physiological, biochemical, and yield-related traits measured at the stem elongation (Table S1) and anthesis stages (Table S2). At the stem elongation stage, gsw and RWC significantly decreased in the wild, landrace, and modern cultivar groups. However, at the anthesis stage, these parameters decreased significantly only in the wild-type group. At the stem elongation stage, proline increased significantly in all the status groups, but there was no significant difference at the anthesis stage. At the stem elongation stage, the SHL decreased significantly in the wild-type group under drought stress, whereas the TT was affected in all the status groups at this stage. However, the ET measured at the maturity stage was not significant for any of the status groups, whereas the PH was significant for both the wild and landrace groups. Under severe drought stress applied at two different stages, we observed significant decreases in yield-related traits, such as GY, BM, and GN, across all the status groups. Among the status groups, only modern cultivars showed a

significant HI response (Table S2).

3.6. Correlations between the stem elongation and anthesis stages for biochemical and physiological traits

We investigated correlations between the stem elongation and anthesis stages related to biochemical and physiological traits (Fig. 4 and Table S3). A weak correlation between the stem elongation and anthesis stages was observed for physiological traits, such as gsw and RWC, and for biochemical traits, such as proline and MDA (Fig. 4). The subsequent analysis revealed positive correlations for gsw ($R^2 = 0.18$, $p < 0.001$), RWC ($R^2 = 0.41$, $p < 0.001$), proline ($R^2 = 0.31$, $p < 0.001$) and MDA ($R^2 = 0.43$, $p < 0.001$), indicating a weak linear relationship and a highly significant association between the two stages. There was more variability in these traits across the two stages for wild taxa than for landraces and modern cultivars. However, no associations were found between the stem elongation and anthesis stages for vegetative indices, such as the NDVI, Lic2, MCARI2, CRI2, and ARI2 (Table S3).

Table 3
Descriptive statistics and analysis of the morphological, phenological, and yield traits of 17 genotypes in the *Triticum* genus.

Traits	Mean (Standard deviation)		ANOVA		
	Well-watered	Drought	Treatment	Genotype	Treatment × Genotype
Total tillers: stem elongation stage	13 (4.3)	8 (3.3)	***	****	ns
Shoot length (cm): stem elongation stage	69.4 (15.0)	56.9 (11.2)	****	****	****
Days to heading	153 (6.8)	150 (7.2)	**	****	ns
Days to anthesis	159 (6.4)	156 (6.2)	**	****	ns
Days to maturity	190 (5.8)	188 (7.8)	ns	****	**
Plant height (cm)	119.1 (23.7)	90.3 (15.6)	****	****	****
Spike length (cm)	10.1 (2.4)	9.4 (2.3)	**	****	****
Effective tillers	17 (8.3)	14 (8.9)	*	****	ns
Peduncle exertion length (cm)	26.4 (10.8)	16.8 (9.1)	**	****	***
Above ground biomass (g)	37.2 (14.8)	18.9 (7.0)	****	****	****
Grain yield (gm)	12.5 (8.3)	6.1 (3.4)	**	****	****
Grain number	450 (232)	242 (121)	**	****	****
Thousand kernel weight (g)	29.8 (13.0)	28.3 (12.6)	ns	****	**
Harvest index (%)	32.1 (11.0)	31.5 (9.1)	ns	****	****

Note: Mean values of all genotypes are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ **** $p < 0.0001$, ns = nonsignificant.

3.7. Associations among the measured traits

We investigated the associations among all the measured traits during the stem elongation stage (Fig. 5) and anthesis stage (Fig. 6) under both well-watered and drought conditions.

3.7.1. Correlations of physiological and biochemical traits with yield-related traits

During the stem elongation stage, none of the physiological or biochemical traits, apart from the NDVI, were associated with yield-related traits under either well-watered or drought stress conditions (Figs. 5A and 5B).

During the anthesis stage, gsw was significantly and negatively correlated with GY and BM under drought stress conditions (Fig. 6B). Similarly, the NDVI was negatively correlated with BM, GY, and TKW under these conditions, but no such correlation occurred under well-watered conditions. Lic2 was significantly and positively correlated with GY and HI under both well-watered and drought stress conditions. In contrast, MCARI1, CRI2, and ARI2 were significantly and negatively correlated with several yield-related traits. For example, MCARI1 was negatively correlated with HI and TKW under well-watered conditions (Fig. 6A) and with BM, GY, HI, and TKW under drought conditions (Fig. 6B). CRI2 was negatively correlated with HI, GY, and TKW under both well-watered and drought conditions. However, other physiological and biochemical traits, such as proline and MDA contents and RWC, were not associated with yield-related traits under either well-watered or drought conditions during the anthesis stage (Figs. 6A and 6B).

3.7.2. Correlations of physiological and biochemical traits

Notably, during the stem elongation stage (Figs. 5A and 5B), gsw was significantly positively correlated with RWC and significantly negatively correlated with the MDA and proline contents under drought stress conditions. However, no such associations were observed under well-watered conditions. Similarly, under drought stress, ARI2 was significantly negatively correlated with the MDA and proline contents but significantly positively correlated with gsw and RWC. These associations were not detected under well-watered conditions. Moreover, no associations were detected between proline and MDA contents during the stem elongation stage under either well-watered or drought conditions.

During the anthesis stage (Figs. 6A and 6B), gsw was significantly negatively correlated with MDA and RWC under well-watered conditions but not under drought conditions. Similarly, under drought conditions, the proline content was significantly negatively correlated with RWC but significantly positively correlated with MDA. Such associations were not observed under well-watered conditions. Under drought conditions, MCARI1 was significantly and positively correlated with gsw but negatively correlated with RWC. However, under well-watered conditions, no associations were detected between gsw or RWC and any of the vegetative indices. In addition, biochemical traits, such as proline and MDA contents, did not exhibit any correlations with vegetative indices under either well-watered or drought conditions.

3.7.3. Correlations of morphological and phenological traits with yield-related traits

TT was significantly negatively associated with TKW at the stem elongation stage under drought stress (Fig. 5B), which was consistent with the finding of a significant negative correlation between ET and TKW at the anthesis stage (Fig. 6B). However, SHL was significantly positively associated with TKW at the stem elongation stage, but no such association was observed between PH and TKW under drought stress.

Notably, SL was positively associated with GY under drought stress but not under well-watered conditions. Similarly, GN was negatively correlated with TKW under drought stress but not under well-watered conditions. PH was negatively correlated with HI under both well-watered and drought stress conditions. PEL was negatively correlated with yield-related traits, such as GY, HI, and TKW, under both well-watered and drought stress conditions. Under drought stress conditions, DTM was positively correlated with GY and BM; however, this association was not detected under well-watered conditions. As we expected, GY was significantly and positively correlated with HI, TKW, and BM under both well-watered and drought stress conditions.

3.8. Principal component analysis

A PCA was performed to investigate associations among various traits and the distributions and associations of the genotypes with these traits. The PCA results revealed that during the stem elongation stage, PC1 explained 31 % of the variation, and PC2 explained 22.3 % of the variation under well-watered conditions (Figure S3A). Under drought stress conditions, PC1 explained 34.1 % of the variation, and PC2 explained 20.3 % of the variation (Figure S3B). At the anthesis stage, PC1 explained 34.2 % of the variation, and PC2 explained 18.3 % of the variation under well-watered conditions (Figure S3C). Under drought stress conditions, PC1 explained 40.4 % of the variation, and PC2 explained 18.9 % of the variation (Figure S3D). Under drought stress, modern cultivars were associated with proline at the stem elongation stage, but no distinct associations were detected under well-watered conditions. At the anthesis stage, the modern cultivars were associated with GY and Lic2 under both well-watered and drought stress conditions. During this stage, the genotypes of wild wheat and modern cultivars were clustered together within each of their status groups under both well-watered and drought stress conditions, whereas the distribution of landraces was scattered. However, at the stem elongation stage, the genotypes were generally more scattered; modern cultivars were an

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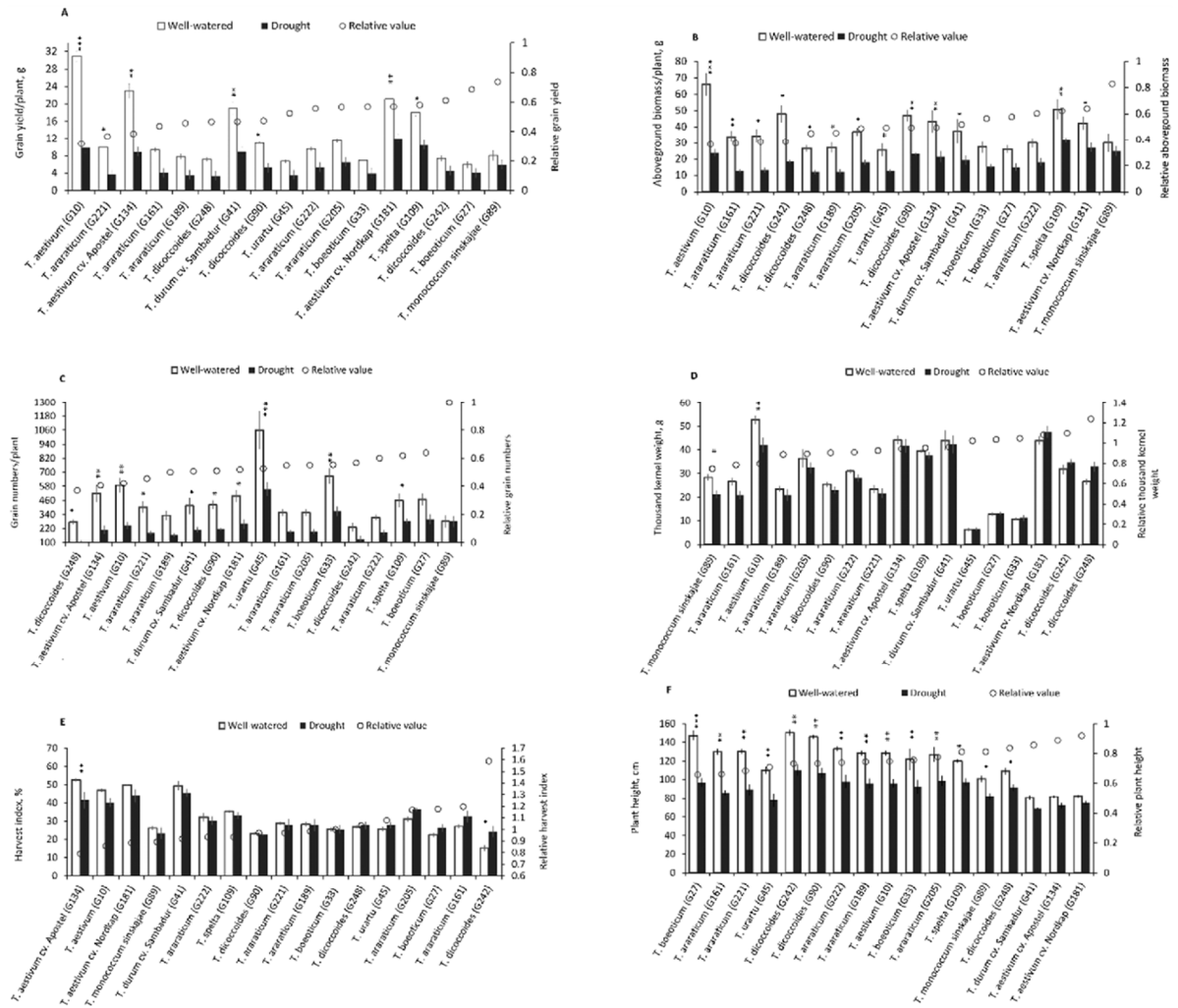


Fig. 3. Grain yield per plant (A), aboveground biomass per plant (B), grain number per plant (C), thousand kernel weight (D), harvest index (E), and plant height (F). The unshaded circles indicate the mean relative values (drought/control) on the secondary vertical axis, and the genotypes are arranged in ascending order from low relative values (left) to high relative values (right). The white and black vertical bars indicate the mean values under well-watered and drought conditions, respectively, on the primary vertical axis. The data are average values of six replicates \pm standard errors. Asterisks above the vertical bar indicate significant differences between the well-watered and drought conditions (treatment effect) within the same genotype according to pairwise comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

exception, as they formed a distinct cluster under drought conditions.

4. Discussion

The high level of genetic diversity found in wild wheat relatives could prove useful in breeding programs (Kilian et al., 2021; Nevo and Chen, 2010; Sharma et al., 2021). In our study, we observed highly significant genotypic variations for all morphophysiological, biochemical, and yield-related traits in the *Triticum* genus that were measured during both the stem elongation and anthesis stages (Tables 2 and 3). Consistent with our results, several studies conducted on the *Triticum* genus have reported substantial genetic diversity for various physiological, morphological, and yield-related traits (Eser et al., 2024; Peleg et al., 2005; Reynolds et al., 2007; Saeidi et al., 2015; Suneja et al., 2019; Wang et al., 2017; Zaharieva et al., 2001). Mguis et al. (2008) reported that the genetic diversity observed for various traits is associated with geographical origin. This finding accords with our observations of

variations in the *Triticum* genotypes used in this study, which were collected from diverse regions. The high genetic variation across the measured traits in the *Triticum* genus offers promising resources for understanding and enhancing drought tolerance in wheat breeding programs.

4.1. Drought stress affects physiological and biochemical traits during the stem elongation and anthesis stages

Our finding that gsw and RWC were significantly reduced due to drought stress during both the stem elongation and anthesis stages (Table 2) is consistent with the findings of previous studies, which reported reductions in gsw and RWC due to drought stress in wheat (Chaouachi et al., 2023; Ru et al., 2023) and maize (Farman et al., 2022). Stomatal regulation is an important first line of response to drought stress in plants. Although a decrease in gsw is considered a drought avoidance strategy for maintaining the RWC, it also limits

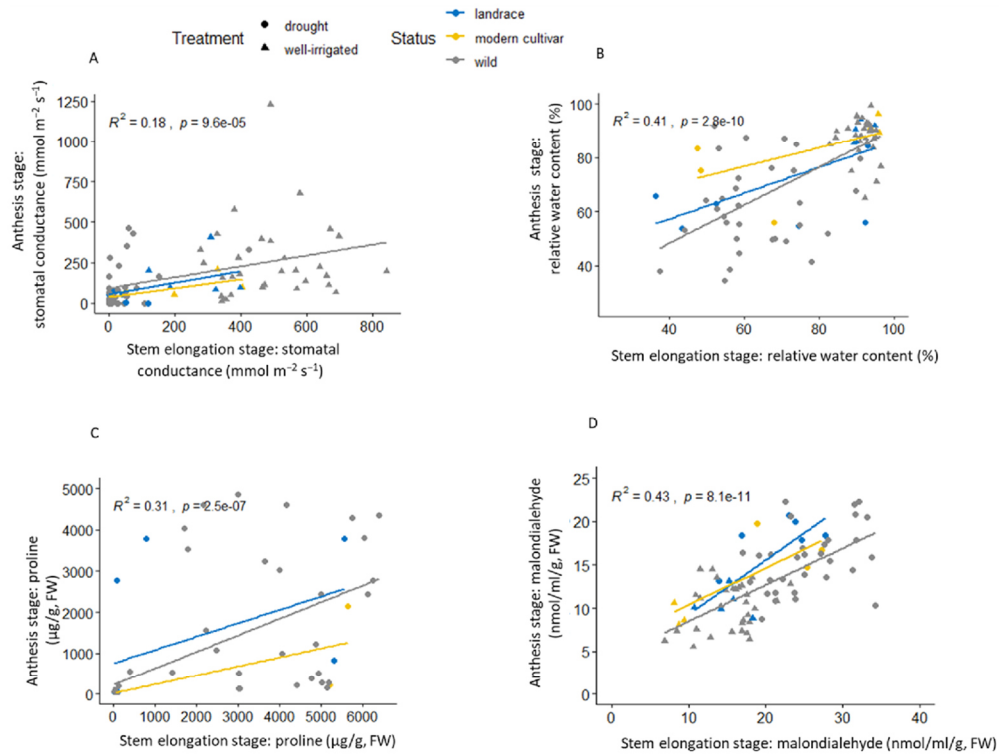


Fig. 4. Relationships between physiological traits at the stem elongation and anthesis stages. Stomatal conductance (A), relative water content (B), proline content (C), and malondialdehyde content (D). The blue, yellow, and gray lines and dots denote the landrace, modern cultivar, and wild status, respectively.

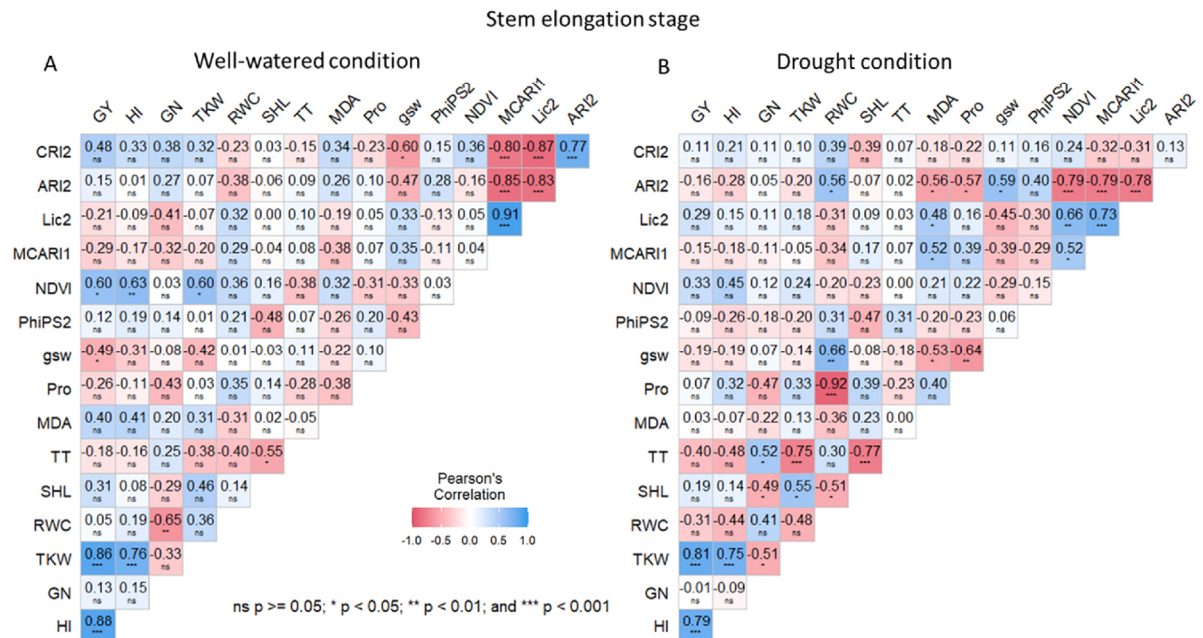


Fig. 5. Pearson's correlations between traits measured during the stem elongation stage under well-watered conditions (A) and drought conditions (B). AR12 = anthocyanin reflectance index 2, CR12 = carotenoid reflectance index 2, GN = grain number, GY = grain yield, gsw = stomatal conductance, HI = harvest index, Lic2 = Lichtenthaler index 2, MCAR1 = modified chlorophyll absorption reflectance index 1, MDA = malondialdehyde, NDVI = normalized difference vegetation index, PhiPS2 = photosystem II efficiency, Pro = proline, RWC = relative water content, SHL = shoot length, TKW = thousand kernel weight, TT = number of tillers.

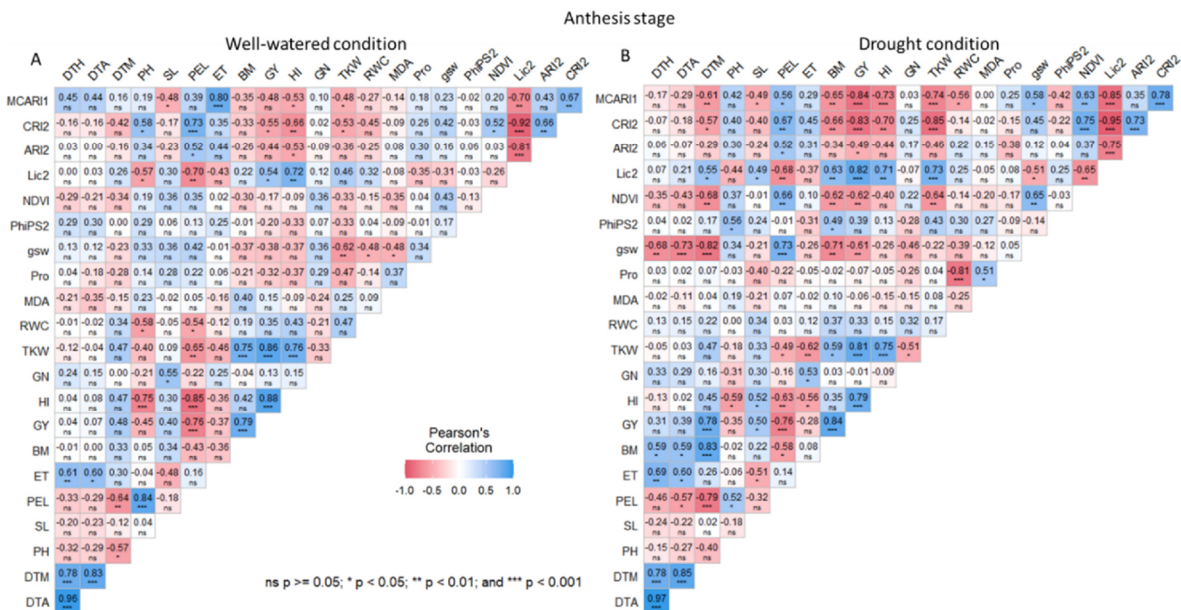


Fig. 6. Pearson's correlations between traits measured during the anthesis stage under well-watered conditions (A) and drought conditions (B). ARI2 = anthocyanin reflectance index 2, BM = aboveground biomass, CR12 = carotenoid reflectance index 2, DTA = days to anthesis, DTH = days to heading, DTM = days to maturity, ET = effective tillers, GN = grain number, GY = grain yield, gsw = stomatal conductance, HI = harvest index, Lic2 = Lichtenhaler index 2, MCARI1 = modified chlorophyll absorption reflectance index 1, MDA = malondialdehyde, NDVI = normalized difference vegetation index, PEL = peduncle exertion length, PhIPs2 = photosystem II efficiency, PH = plant height, Pro = proline, RWC = relative water content, SL = spike length and TKW = thousand kernel weight.

photosynthesis (Li et al., 2017). Moreover, the accumulation of MDA and proline contents (Table 2) can be interpreted as biochemical markers of drought stress (Kamruzzaman et al., 2023; Shrestha et al., 2022). During both the stem elongation and anthesis stages, the majority of the genotypes in this study demonstrated tolerance to oxidative damage. MDA is a lipid peroxidation product that indicates the level of oxidative damage in plants (Quagliata et al., 2023; Sallam et al., 2019). During the stem elongation stage, most of the genotypes showed significant accumulation of proline rather than MDA, suggesting that proline may have acted as an antioxidant. However, *T. durum* cv. Sambadur (G41) and *T. araraticum* (G221) revealed significant increases in both the MDA and proline contents, indicating increased stress levels in these genotypes. Therefore, our results revealed a genotype-dependent response to oxidative stress and proline accumulation. Proline accumulation in plants plays an important role in scavenging against oxidative stress, while also facilitating osmotic adjustment and stabilizing proteins and cell structures (Bandurska, 2022; Kavi Kishor and Sreenivasulu, 2014).

Plants experience osmotic stress when exposed to drought conditions. Therefore, maintaining a stable water content is essential for their optimal growth and development. We observed significant and negative correlations between proline content and RWC during both the stem elongation and anthesis stages under drought stress (Figs. 5B and 6B), which is in accordance with findings reported in previous studies (Marček et al., 2019; Saghour el idrissi et al., 2023). Furthermore, increased proline accumulation occurs in tissues with low water contents as opposed to those with high water contents (Chiang and Dandekar, 1995). Consequently, the high accumulation of proline in some drought-sensitive genotypes may be a symptom of stress rather than simply reflecting osmotic adjustments (Sanchez et al., 2012). Other researchers have argued that high levels of proline accumulation are linked to drought tolerance (Quagliata et al., 2023; Shrestha et al., 2022). However, our results revealed a significant treatment × genotype interaction for proline content and RWC during both the stem elongation

and anthesis stages (Table 2), indicating variations in genotypic responses to the treatment effects for these traits. These findings indicate that some genotypes effectively regulate proline accumulation and maintain RWC under drought stress conditions.

4.2. Assessing genotypic variations in response to drought stress during the stem elongation and anthesis stages

Stomatal closure helps plants survive severe drought stress over longer periods (Gallé et al., 2013; Onyemaobi et al., 2021; Sade et al., 2012). However, not all the genotypes showed a significant decrease in gsw during the anthesis stage. Some shifts were observed during the anthesis stage, particularly in the *T. araraticum* (G161 and G189) genotype, which presented greater relative changes than during the stem elongation stage (Figs. 1A and 1B). Interestingly, these genotypes transitioned from being water-saving (isohydric) to becoming water-wasting (anisohydric) between the two stages, showing dehydration tolerance despite a significant decrease in RWC. This mechanism enables the maintenance of carbon gain during anthesis and grain filling, but it could increase the risk of hydraulic failure under severe drought stress (Scholz et al., 2012). The significant negative correlation between gsw and MDA under drought stress conditions during the stem elongation stage indicates that stomatal closure limits CO₂ assimilation, which triggers excessive oxidative stress in plants. However, no such association was observed during the anthesis stage, which may be attributed to the signaling effect that develops during the stem elongation stage. However, the reason for this phenomenon remains unclear.

Not all the genotypes responded significantly to drought stress in terms of RWC at both stages (Figs. 1C and 1D). Previous studies have shown that drought-tolerant cultivars exhibit a lower decrease in RWC than sensitive cultivars do (Cheng et al., 2016). In light of our results, the *T. monococcum* subsp. *sinskajae* (G89), *T. urartu* (G45), *T. araraticum* (G205), and *T. boeoticum* (G33) genotypes could be considered drought tolerant in terms of RWC at the stem elongation stage. *T. boeoticum*

(G27) revealed the highest level of drought tolerance, and only a few genotypes presented a significant decrease in RWC at the anthesis stage. Furthermore, *T. aestivum* (G10) showed the greatest susceptibility to RWC at the stem elongation stage, which significantly affected SHL and TT. Consequently, yield-related traits, namely, GY, BM, GN, and TKW, were also significantly affected in this genotype, which was therefore considered drought-susceptible. During the stem elongation stage, *T. araraticum* (G222) exhibited the lowest degree of response to drought stress for MDA, whereas *T. aestivum* cv. Nordkap (G181) showed the lowest degree of response during the anthesis stage, indicating avoidance of oxidative stress. During both stages, drought stress significantly increased the MDA content in *T. durum* cv. Sambadur (G41), indicating its susceptibility to oxidative stress. Previous studies have reported that wild wheat species such as *T. dicoccoides*, *T. boeoticum* and *T. urartu* are candidates for drought tolerance (Pour-Aboughadareh et al., 2017; Suneja et al., 2017). However, those studies did not address the impact of consecutive drought stress during both the vegetative and reproductive stages in wild wheat relatives. Furthermore, abiotic stress tolerance traits, including stomatal conductance, have rarely been investigated in *T. araraticum*. To our knowledge, only Sultan et al. (2012) included a single genotype of *T. araraticum* for studying physiological traits such as the MDA content, RWC and proline content and reported that it is susceptible to drought stress. In contrast, in our study, some genotypes of *T. araraticum* showed dehydration tolerance, indicating large variation in adaptability within *T. araraticum*.

Our results revealed a notable decrease in the relative value of MDA in *T. araraticum* (G221) and *T. boeoticum* (27) during the anthesis stage compared to the stem elongation stage. Preexposure of plants to environmental stresses can increase their ability to activate their defense mechanisms rapidly upon subsequent experiences of stress (Martinez-Medina et al., 2016). The decrease in MDA during the second drought episode suggested improved redox defense in the plants, which was likely due to the downregulation of cell membrane lipid peroxidation, which mitigated oxidative stress under drought stress (Abid et al., 2016). However, direct comparison of separate drought priming episodes during the two stages was limited by our experimental design: The same plants were subjected to drought treatments during both drought episodes, thereby confining our comparison of drought stress responses to the well-watered conditions at both stages. Our results revealed that, with a few individual exceptions, drought stress induced a lower increase in proline accumulation during the anthesis stage than during the stem elongation stage. Similar results have been reported for sugar beet, with a decrease in proline accumulation observed during subsequent drought episodes compared with its accumulation during initial stress events (Leufen et al., 2016).

During the stem elongation stage, ARI2 was negatively associated with MDA under drought stress (Fig. 5B, Figure S3B). Anthocyanins protect leaves from excess light and serve as scavengers of reactive oxygen species (Sims and Gamon, 2002). The increased efficiency of plant photosystems under drought stress helps prevent oxidative damage (Abid et al., 2016). This effect was also demonstrated by the positive association between ARI2 and RWC in our results (Fig. 5B, Figure S3B), indicating that photoprotection may influence plant hydration. Moreover, we found a weaker significant positive association between the stem elongation and anthesis stages for biochemical and physiological traits, excluding vegetative indices (Fig. 4, Table S3). The response of plants to drought stress is influenced by their growth stage, age, species, or genotype and by the duration of drought stress (Gray and Brady, 2016). Therefore, the physiological and biochemical mechanisms involved in drought tolerance remain complex. Moreover, the trait associations in this study may have been masked by the high genotypic variation. Our results suggest genotype-dependent responses to drought stress across the developmental stages of plants.

4.3. Comparative analysis of drought adaptation mechanisms in wild wheat relatives and modern wheat cultivars

In our study, proline accumulation at the anthesis stage did not significantly differ according to treatment effect across status groups (Table S2). Our results revealed significant treatment effects for RWC and MDA in the wild-type group but no clear trait dominance favoring the wild-type status for drought tolerance. Instead, we observed a genotype-specific response for these traits (Figs. 1D and 2B). However, a previous study (Wang et al., 2017) reported stronger biochemical responses to drought tolerance in modern hexaploid cultivars than in landraces and wild wheats, but that study included different sets of genotypes from domesticated *Triticum* and wheat wild relatives belonging to *Aegilops tauschii*.

Our findings of greater reductions in PH and gsw under drought stress in wild plants and landraces (Table S2) indicate that drought avoidance strategies are more effective in wild wheat relatives and landraces than in modern cultivars. The significant negative correlation between gsw and GY under drought stress in this study indicated that drought avoidance contributes positively to GY. This finding contradicts previous findings of a positive association between gsw and GY under stress (Fischer et al., 1998; Zheng et al., 2011). A meta-analysis (Li et al., 2021) revealed that drought tolerance via osmotic adjustment and the antioxidant defense system did not lead to increased yield under severe drought stress in wheat, which is consistent with our finding of no correlation between RWC or proline content and GY or other yield-related traits. These findings suggest that complex physiological and biochemical mechanisms contribute to the GY under severe drought stress. These may have low heritability (Chen et al., 2012) and therefore low breeding value. Although no correlation was detected between proline content and yield (Fig. 5 and Fig. 6), the drought-tolerant genotypes reported in our study possibly benefited from drought-induced proline accumulation, which occurred in all the studied genotypes under drought stress (Figs. 2C and 2D).

We observed accelerated heading, anthesis, and maturity (Table 3). Phenological adaptation is a drought escape mechanism and an important factor influencing GY in plants (Foulkes et al., 2007; Shavrukov et al., 2017). Plants with longer phenological durations are prone to drought stress. A short anthesis and grain-filling period induced by drought stress reduces GY in wheat (Eser et al., 2024; Lv et al., 2017). However, we observed a yield advantage due to early anthesis induced by drought stress in certain genotypes. For example, the *T. dicoccoides* (G242) genotype exhibited a drought escape mechanism via early anthesis under drought stress but did not significantly reduce GY or GN; instead, it significantly increased HI. Additionally, *T. monococcum* subsp. *sinskajae* (G89) and *T. boeoticum* (G27) revealed a drought escape mechanism. These findings suggest that these genotypes may have employed drought escape mechanisms to mitigate the negative effects of drought stress, while they activated the physiological grain formation process after watering recommenced to maintain the GY under drought stress. In line with previous findings (Lopes et al., 2012), we found a positive correlation between DTM and GY under drought stress, but no correlation was detected between DTH or DTA and GY, averaged across all the genotypes. These findings reveal a complex interplay between phenological responses and yield components under water stress in the *Triticum* genus. To some extent, our observations of responses to drought stress across the status groups are aligned with those of a previous study (Li et al., 2021), which showed that some wild wheat relatives and landraces in the *Triticum* genus exhibit drought avoidance and escape mechanisms. Consequently, breeding for drought adaptation in wheat should also incorporate avoidance and escape mechanisms together with drought tolerance (Mohammadi, 2018; Sallam et al., 2019). In our study, *T. boeoticum* (G27) demonstrated all three mechanisms for drought adaptation: drought escape, avoidance, and tolerance via enhanced anthesis; a high response to gsw and PH; and a low reduction in RWC at the anthesis stage. Similarly, *T. monococcum* subsp. *sinskajae*

(G89) exhibited a drought escape mechanism via early anthesis, and among the genotypes with a lower reduction in RWC, it demonstrated drought tolerance at the stem elongation stage. *Triticum dicoccoides* (G242) evokes a drought escape mechanism via enhanced anthesis and drought tolerance via high RWC at the anthesis stage. In our previous field experiment, *T. dicoccoides* (G242) and *T. boeoticum* (G27) also showed drought adaptation, with high responses to gsw, and *T. monococcum* subsp. *sinskajae* (G89) exhibited a low response to gsw while maintaining yield under rainfed conditions (Pantha et al., under review). The underlying drought adaptation in these genotypes can be attributed to the distinct semiarid to arid climates of their native regions in the Fertile Crescent, characterized by hot, dry summers and water scarcity, which pose key environmental constraints (Jaradat, 2017). These genotypes can be utilized as sources of drought adaptation traits. In our experiment, not all the wild genotypes and landraces showed drought tolerance; however, the drought-tolerant candidates identified were primarily from wild wheat and landrace groups, which were sourced from our own collection and seed multiplication efforts. Modern wheat varieties exhibit narrow genetic diversity for drought tolerance; therefore, wild wheat relatives and landraces may be promising natural sources for additional drought tolerance genes and mechanisms (Sharma et al., 2021). Therefore, this study is highly relevant for biological research and food security. Moreover, this study highlights the importance of integrating different drought adaptation mechanisms to achieve stable yields under severe and frequent drought stress, particularly at the stem elongation and anthesis stages. Previous studies have emphasized that drought tolerance aligns with yield (Blum, 2017; Wang et al., 2017). However, they did not account for the contribution of drought avoidance mechanisms to yield under severe drought stress. Our findings showed that drought avoidance can possibly contribute to yield in specific genotypes under severe and frequent drought stress conditions. Therefore, the potential ability of a specific genotype to adapt to varying degrees of drought stress should be emphasized (Turner et al., 2007).

We used *T. dicoccoides* genotypes from two different genetic taxa: *T. dicoccoides* (G242), a western race from Israel, and *T. dicoccoides* (G90 and G248), a central-eastern race from Turkey and Iran (Özkan et al., 2011). In our study, the western race presented greater tolerance at anthesis, whereas the central-eastern race presented greater tolerance at the stem elongation stage, with relatively greater RWC.

4.4. Morphological and yield component plasticity in the investigated genotypes

Our results revealed that drought stress negatively impacted morphological and yield-related traits, notably PH, SL, PEL, ET, BM, GY, and GN (Table 3). Among these traits, GY, averaged across all the genotypes, presented the greatest reduction. Our results revealed a significant reduction in the GY of 51 %, which is consistent with a previously reported decrease in yield of 53 % in wheat under severe drought stress (Ahmed et al., 2020). In light of the relative performance of GY in our study, the *T. monococcum* subsp. *sinskajae* (G89), *T. boeoticum* (G27), and *T. dicoccoides* (G242) genotypes showed the greatest drought tolerance. Furthermore, the maintenance of GY under drought stress in these genotypes was attributed to various yield components and morphological traits, each contributing differently to different genotypes. The drought tolerance of cereal crops is characterized primarily by their ability to maintain stable yields under drought stress conditions and how they respond to each yield component (Bandurska, 2022; Wang et al., 2017). Decreased competition for resources allocated, for example, to PH and BM, could increase the allocation of resources to spikes, which would contribute to increasing the HI (Foulkes et al., 2011). Our results revealed a significant negative correlation between PH and HI, which suggests a trade-off between the allocation of resources for PH and grain sink strength. On the one hand, a drought-tolerant genotype, *T. dicoccoides* (G242), showed significantly decreased PH and BM and consequently a significantly increased HI,

although the GN decreased significantly. The drought-tolerant genotypes *T. monococcum* subsp. *sinskajae* (G89) and *T. boeoticum* (G27) stabilized GY through the maintenance of GN. Grain number and seed weight are related to grain sink strength (Miralles and Slafer, 2007; Sadras and Slafer, 2012; Slafer et al., 2023). Our results indicated that GN was negatively correlated with TKW, suggesting a trade-off between resource allocation for grain size and grain number, as reported by Quintero et al. (2018).

The limited tillering ability of plants under water stress conditions is advantageous for efficiently conserving water and could contribute to increased yield-related traits (Richards et al., 2010). This is supported by our finding of a significant negative correlation between TT and TKW during the stem elongation stage under drought stress conditions. Furthermore, we observed a significant negative correlation between ET and HI with TKW only under drought stress conditions. However, SL was positively correlated with GY under drought stress conditions but not under well-watered conditions. Chen et al. (2012) reported that SL is one of the major traits for drought tolerance and contributes to GY in wheat under water stress conditions. Therefore, our results confirm that reduced PH and tillers may be advantageous for efficient water conservation during the grain-filling stage and highlight the significant contribution of SL to maintaining GY under severe drought stress conditions.

In general, climate-controlled greenhouse pot experiments are used to study complex drought tolerance mechanisms accurately, minimizing external factors to ensure consistent results (Poorter et al., 2016). However, they limit root growth, which is a key factor for drought tolerance, and other external environmental factors that occur under field conditions. Therefore, we propose further confirmation in field conditions with their inherent variability, including fluctuating environmental factors and complex interactions.

5. Conclusion

Genotype-dependent responses to drought were observed during the stem elongation and anthesis stages, with some genotypes exhibiting drought tolerance during both stages. Our first hypothesis that drought tolerance mechanisms for osmotic and stomatal regulation are correlated between the stem elongation and anthesis stages was partially confirmed by the results. Certain genotypes that adapt to drought through different mechanisms were able to maintain yield stability under drought stress, although traits such as proline content, RWC and MDA content were not associated with GY, which might be due to the highly variable genotypes used in this study. The results related to our second hypothesis, namely, that wild wheat relatives exhibit superior drought tolerance compared with modern wheat cultivars, were inconclusive. We observed genotype-dependent responses to drought stress, revealing trait diversity in the studied *Triticum* gene pool and identified candidates for drought tolerance, which can be utilized in wheat breeding programs. These candidates exhibit specific physiological and biochemical traits that could increase drought tolerance, contributing to yield. Therefore, exploiting different mechanisms of drought adaptation, such as drought avoidance, drought escape, and drought tolerance, in breeding programs is crucial for enhancing yield stability under drought conditions. Our study contributes to a substantial expansion of the gene pool that can provide traits in wheat improvement programs aimed at drought adaptation.

Funding statement

This work was supported by the German Academic Exchange Service (DAAD) under the Programm des Projektbezogenen Personenaustauschs (PPP) Project [project number 57627943] and the Biodiversity for Opportunities, Livelihoods and Development (BOLD) Project funded by the Government of Norway [grant number: QZA-20/0154]. SP received a PhD scholarship from DAAD. The funders did not have any role in the

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study design or implementation.

CRedit authorship contribution statement

Sumitra Pantha: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Benjamin Kilian:** Writing – review & editing, Conceptualization. **Hakan Özkan:** Writing – review & editing, Conceptualization. **Frederike Zeibig:** Writing – review & editing, Investigation, Conceptualization. **Michael Frei:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Sumitra Pantha reports financial support was provided by German Academic Exchange Service. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to Liane Renno, Erika Schick, and Emily Siegwald for their assistance during the greenhouse experiments; laboratory work; and the threshing, cleaning, and measuring of the samples. The authors would also like to thank Udo Schnepf and Till Strobusch, who helped set up the greenhouse growing conditions. Finally, they would like to thank Radhika Johari for her help in editing the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.envexpbot.2024.106047](https://doi.org/10.1016/j.envexpbot.2024.106047).

Data availability

All data relevant to this article can be found in the article and in the supplementary material.

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4 A comparative analysis of drought tolerance mechanisms in *Triticum araraticum* Jakubz. and modern bread wheat (*Triticum aestivum* L.) cultivars

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Journal of Agronomy and Crop Science (under review)

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Figures: Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5

Supporting information (provided in additional files): Supporting information Fig. S1-S5 and supporting information Table S1.

Abstract

Wild wheat relatives are promising sources of drought tolerance that demonstrate adaptive mechanisms not found in modern wheat cultivars. This study investigated and compared the drought tolerance mechanisms in *Triticum araraticum* genotypes, a relatively unexplored species, with those of modern *Triticum aestivum* cultivars. Osmotic regulation, antioxidants, and gas exchange traits were evaluated under well-watered and drought treatments. Overall, high-yielding bread wheat cultivars exhibited a higher photosynthetic rate and instantaneous water use efficiency, and greater antioxidant activity, with a few exceptions compared with *T. araraticum* under the well-watered treatment. Drought stress significantly reduced stomatal conductance, internal CO₂ concentration, and photosystem II efficiency. It induced a significant increase in the content of proline, total soluble sugars, and most enzymes involved in the ascorbate–glutathione cycle, except for ascorbate peroxidase and ascorbate oxidase, which remained unresponsive. *T. araraticum* genotypes demonstrated a more pronounced drought response with distinct antioxidant tolerance mechanisms compared with modern *T. aestivum* cultivars. Specifically, in *T. araraticum*, the increase in the redox state of glutathione, driven by enhanced glutathione reductase activity, was higher than that of ascorbate under drought stress. By contrast, *T. aestivum* maintained increased activity of guaiacol peroxidase and enhanced ascorbate redox state through dehydroascorbate reductase. This study provides valuable insights into the unique antioxidant and osmotic regulator of *T. araraticum* that differ from those of modern bread wheat cultivars. Its findings can inform future wheat improvement programs aimed at developing climate-resilient wheat cultivars for sustainable agricultural systems.

Keywords

Antioxidant, gas exchange, drought, osmotic adjustment, *Triticum*

Highlights

- Drought stress enhanced water use efficiency, proline, sugars, and antioxidants.
- *Triticum araraticum* showed drought tolerance mechanisms not seen in modern wheat.
- Unlike modern wheat, *T. araraticum* relied on the glutathione redox balance for its stress response mechanisms.
- Photosynthesis, water use efficiency, and antioxidants were superior in modern wheat under well-watered conditions.

1. Introduction

Drought stress severely affects plant growth and development, posing a significant challenge to crop productivity. An understanding of the complex mechanisms underlying plant responses to drought stress is critical for enhancing crop productivity in drought-prone regions (Farooq et al., 2009; Sallam et al., 2019). Therefore, there is an urgent need to explore plant genetic resources for traits conferring abiotic stress tolerance to enhance the useful genetic diversity of wheat breeding pools (Leigh et al., 2022; Nevo and Chen, 2010).

Drought stress inhibits photochemical reactions and reduces photosynthetic efficiency (Akula et al., 2024). It also disrupts the balance of reactive oxygen species (ROS) in plant cells, leading to metabolic changes and ROS accumulation (Naderi et al., 2020). Excessive ROS production can damage proteins, lipids, and DNA, inducing oxidative stress (Mittler, 2006).

To protect themselves against oxidative damage, plants deploy a range of antioxidant defenses. Enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (POD), ascorbate oxidase (AO), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR). Non-enzymatic antioxidants, such as ascorbate (AsA) and glutathione (GSH), directly detoxify ROS (Apel and Hirt, 2004; Asada, 1999). For instance, SOD mitigates oxidative damage by converting superoxide into H_2O_2 (Frei et al., 2010), which is subsequently detoxified via the ascorbate–glutathione (AsA–GSH) cycle involving APX, GR, and related enzymes (Asada, 1999; Wu et al., 2021).

Osmotic adjustment (OA) is another critical drought tolerance mechanism, enabling plants to accumulate compatible solutes, like proline and soluble sugars, to maintain turgor and metabolic functions under low water potential (Blum 2017; Ru et al., 2023). Proline also mitigates lipid peroxidation inhibition, scavenges ROS, and prevents cell damage (Hayat et al., 2012). Drought-tolerant plants typically exhibit higher levels of osmoprotectants and robust antioxidant defense mechanisms (Farooq et al., 2009; Sallam et al., 2019).

Determination of plant water status and gas exchange parameters is essential for analyzing the effect of drought stress physiology in wheat (Pflüger et al., 2024). Drought stress reduces the water status of plants, including their leaf water potential and relative water content (Farooq et al., 2009). Consequently, it closes stomata, lowering the transpiration rate and CO_2 uptake, resulting in reduced photosynthesis (Farooq et al., 2024).

Wild wheat relatives harbor potentially valuable genetic resources for conferring drought tolerance, displaying traits such as efficient stomatal regulation, osmotic adjustment (OA),

and antioxidant responses (Leigh et al., 2022; Suneja et al., 2019). Among the tetraploid wild wheat species, while *Triticum dicoccoides* (Körn. ex. Asch. & Graebn.) Schweinf. has been extensively studied for its drought tolerance traits, *Triticum araraticum* Jakubz. remains underexplored (Leigh et al., 2022; Pantha et al., 2025). The findings of a recent study suggest that *T. araraticum* exhibits promising traits for enhancing wheat resilience to drought (Pantha et al., 2024). However, its antioxidant defense and gas exchange physiology under drought stress remain largely unknown. This study investigated drought tolerance mechanisms in *T. araraticum* genotypes, focusing on antioxidants, osmotic regulators, and gas exchange traits, which were subsequently compared with those of modern bread wheat (*T. aestivum* L.) cultivars. We hypothesized that (i) modern bread wheat cultivars exhibit a high photosynthetic rate and antioxidant activity under well-watered conditions; and (ii) *T. araraticum* displays a more pronounced drought response, characterized by enhanced osmotic adjustments and greater antioxidant activity. The findings of this study on the drought tolerance mechanisms in *T. araraticum* provide insights for improving wheat's resilience to drought stress and developing wheat varieties that are better suited to drought-prone environments.

2. Materials and methods

2.1. Plant materials

Five genotypes from two different *Triticum* species: three genotypes (G205, G221, G222) of *T. araraticum* (GGA^tA^t) and two modern bread wheat cultivars (Apostel and Nordkap) were used as the experimental material (Table S1). These genotypes were selected from an extensive *Triticum* gene pool previously studied for drought tolerance traits (Pantha et al., 2024). This study builds upon our previous findings (Pantha et al., 2024, 2025) by selecting three potentially drought-tolerant genotypes of *T. araraticum* to investigate their underlying tolerance mechanisms, while the two modern bread wheat cultivars served as controls for comparison.

2.2. Experimental conditions and treatments

The experiment was conducted in a greenhouse (October 2023 to May 2024) at Justus Liebig University, Germany. A minimum photosynthetically active photon flux density (PPFD) of 300 $\mu\text{molm}^{-2}\text{s}^{-1}$ was maintained throughout the experiment using both natural and artificial lighting. Seeds were germinated and plants were grown in N-type soil substrate (Hawita Gruppe GmbH, Germany), following the procedure described by Pantha et al. (2024), with slight modifications. When plants reached Z12 stage (Zadoks et al., 1974), they were transferred to a vernalization chamber maintained at 4 °C for about 2 months. Plants were then transplanted into 5 L plastic pots and each pot contained one plant, with eight

replicates per genotype arranged in a randomized complete block design with two treatments: well-watered and drought stress. After acclimatization for two weeks as described in Pantha et al. (2024), the growth conditions were maintained by a 16 h/8 h light/dark cycle with day/night temperatures of 22 °C/17 °C until crop harvest.

Soil moisture content was monitored using an HH2 soil moisture meter (Delta-T Devices Ltd., Cambridge, UK, version 4.2; Fig. S1), as described by Pantha et al. (2024). Soil moisture content in well-watered plants was maintained >80 % of the pot's soil water-holding capacity (PWHC) throughout the growth period. The drought stress was imposed 70 days after seedling transplanting, at which point the *T. araraticum* genotypes had reached the inflorescence emergence stage (Z50), while the *T. aestivum* genotypes were at the booting stage (Z40). The stress period lasted for 10 days, during which time the soil moisture was reduced to <10 % of the PWHC. Leaf water status was assessed on the 10th day of the drought period. Following the collection of physiological measurements and the harvesting of fresh samples for biochemical analysis 10 days after the drought treatment, the drought-stressed plants were rewatered to restore soil moisture comparable to those in the well-watered treatment. Following full ripening, whole shoots, including spikes, were harvested to evaluate yield-related traits.

2.3. Measurement of leaf water potential and osmotic potential

Leaf water potential- ψ_w (WP) was determined in a freshly harvested leaf using a Scholander bomb (Model 1505D, PMS Instrument Co., Albany, OR 97322, USA) according to Scholander et al. (1965). Osmotic potential- ψ_s (OP) was measured following the method described by Amede et al. (2004). The sap was extracted by pressing the frozen leaf in a syringe and its concentration (mOsmol) was determined using a freezing point osmometer (K-7400S Semi-Micro Osmometer, KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany). Osmotic potential was calculated using Van't Hoff's equation as follows:

$$OP (\psi_s, \text{MPa}) = \text{mOsmol} \times \text{gas constant} (0.831 \times 10^{-5} \times \text{Temperature (K)}) \quad (1)$$

where, OP (ψ_w , MPa) denotes the osmotic potential expressed in megapascals and mOsmol is the solute concentration of the sap, and K is the temperature constant.

2.4. Relative water content and absolute water content measurement

The relative water content (RWC) of the leaves was measured according to Shrestha et al. (2022) and described in detail in Pantha et al. (2024). Briefly, fresh weight (FW) was recorded immediately and turgid weight (TW) was recorded after dipping the leaf section in deionized water for 24 h. Dry weight (DW) was measured oven drying it at 80 °C for 48 h. RWC and absolute water content (AWC) were computed as:

$$RWC = (FW - DW)/(TW - DW) \times 100 \quad (2)$$

$$AWC = (FW - DW)/DW \quad (3)$$

2.5. Measurement of gas exchange and chlorophyll fluorescence

Gas exchange and chlorophyll fluorescence were measured on the flag leaf of the main tiller on each plant. The net photosynthetic rate (P_n), transpiration rate (E) and internal CO_2 concentration (C_i) were measured using a LI-6800 portable photosynthetic system (LI-COR, Inc., Lincoln, Nebraska, USA) 7 days after the drought treatment in four replicates per genotype per treatment. Measurement was conducted at a constant PPFD of $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, a leaf temperature of 22°C , a CO_2 reference value of 400 ppm, a flow rate at $300 \mu\text{mol/s}$, and 60 % relative humidity. The instantaneous photosynthetic water use efficiency (WUE_{leaf}) was calculated as:

$$WUE_{\text{leaf}} = P_n/E \quad (4)$$

Stomatal conductance (g_{sw}) and the quantum efficiency of photosystem II (Φ_{PS2}) were measured, averaged from repeated measurements taken 5, 7, 8, and 10 days after the drought treatment using a portable handheld device (LI-600; LI-COR, Lincoln, Nebraska, USA) in six replicates per genotype in each treatment. Gas exchange parameters were recorded between 9:00 h and 16:00 h.

2.6. Stomata counting and chlorophyll content

The stomata density and length were assessed on the adaxial surface of the top fully expanded leaf using nail polish imprints transferred to slides, and examined under a fluorescence microscope (Nikon ECLIPSE E600; Nikon, Japan). Images were analyzed using NIS-elements software (Nikon, version 5.21.00). Stomata density (SD) was calculated as the number of stomata per mm^2 field of view, and stomata length (SZ) was measured for 10 randomly selected stomata per image.

SPAD was measured using a portable spectrometric device (SPAD-502Plus; Konica Minolta, Inc. Tokyo, Japan) in six replicates per genotype per treatment at the end of the drought stress treatment.

2.7. Biochemical analysis

Two to three of the top leaves were collected from four replicates per genotype per treatment, and immediately were stored at -80°C for subsequent biochemical analyses. The absorbance for all measured biochemical analysis was determined using a microplate reader (Infinite 200 Pro, Tecan, Groedig, Austria).

2.7.1. Lipid peroxidation assay

Lipid peroxidation was determined by the malondialdehyde (MDA) concentration according to Ali et al. (2019). Briefly, MDA was extracted from fresh tissue (100 mg) using 1.5 mL of 0.1 % (w/v) trichloroacetic acid (TCA) and centrifuged at $14,000 \times g$ for 15 min at 4 °C. The reaction mixtures comprised 500 μL of aliquots combined with same volume of reaction solutions as described in detail in Shrestha et al. (2022). The absorbance was determined at 440, 532, and 600 nm.

2.7.2. Analysis of non-enzymatic antioxidants and osmolytes

Reduced and oxidized ascorbate (AsA) from the leaf tissue were determined using the spectrophotometer method introduced by Ueda et al. (2013) and described in detail in Feng et al. (2023). Total and reduced GSH were determined using the method described by Frei et al. (2010).

Proline content was analyzed according to Bates et al. (1973), and described in detail in Pantha et al. (2024).

Total soluble sugar (TSS) content was measured using the phenol-sulfuric acid method according to Dubois et al. (1956), with modifications. Fresh sample (50 mg) homogenized in 1.5 mL ethanol (80 %) was heated in a water bath (80 °C for 30 min) and then centrifuged at $10,000 \times g$ for 10 min. Supernatant was evaporated to dryness using a thermal shaker at 70 °C, and the left-over pellet was re-dissolved with 2 mL water. The reaction mixture comprising 200 μL of sample extract, 200 μL of 5 % phenol, and 1 mL of concentrated H_2SO_4 was incubated at room temperature for 10 min. The absorbance was measured at 490 nm.

2.7.3. Enzyme activity assays

The activities of enzymes, namely APX (EC 1.11.1.11), MDHAR (EC 1.6.5.4), and GR (EC 1.6.4.2) were measured using the same extract prepared following the method described by Wu et al. (2021). Briefly, extract was obtained by homogenizing plant sample (100 mg) in 1 mL of 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.8) containing 1 mM EDTA and 1 mM AsA, and then centrifuged at $10,000 \times g$ for 30 min at 4 °C. For measuring APX activity, reaction mixture comprising 80 μL of 100 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 6.8) with 0.6 mM AsA, 10 μL of 0.03 % H_2O_2 , and 10 μL of sample extract was monitored at 290 nm for 3 min ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$). To measure MDHAR activity, reaction mixture of 10 μL of plant extract, 80 μL of 50 mM Tris-HCl buffer (pH 7.6) containing 2.5 mM AsA, 0.125 units of AO and 10 μL of 1 mM NADH was monitored at 340 nm for 3 min ($\epsilon = 6.2 \text{ mM}^{-1} \text{ cm}^{-1}$). Similarly, for measuring GR activity, a mixture of 10 μL of plant extract, 80 μL of 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.8)

containing 0.5 mM oxidized glutathione (GSSG) and 10 μL of 1.2 mM NADPH was monitored at 340 nm for 3 min ($\epsilon = 6.2 \text{ mM}^{-1} \text{ cm}^{-1}$).

DHAR activity (EC 1.8.5.1) and AO activity (EC 1.10.3.3) were measured following the procedure described by Wu et al. (2021). For DHAR and AO activity, the absorbance kinetics were measured at 265 nm for 10 min ($\epsilon = 14.3 \text{ mM}^{-1} \text{ cm}^{-1}$) and 15 min ($\epsilon = 14.3 \text{ mM}^{-1} \text{ cm}^{-1}$) respectively.

POD activity (EC 1.11.1.7) was measured according to Frei et al. (2010). The reaction mixture (100 μL) consisted 10 μL of the extract, 50 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (pH 7.0), 4 mM guaiacol, and 10 mM H_2O_2 . POD activity was determined by the increase in absorbance at 470 nm for 3 min ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$).

Total SOD activity (EC 1.15.1.1) was determined according to Frei et al. (2010), with slight modifications. Plant sample (100 mg) was homogenized in 1.5 mL of 50 mM HEPES buffer (pH 7.6) containing 0.1 mM EDTA and centrifuged at $15,000 \times g$ for 15 min at 4 °C. Reaction mix (1 mL) containing 10 μL of enzyme extract, 2 μM of riboflavin, 13 mM of methionine, 5 mM of Na_2CO_3 (pH 10.4) and 75 μM of nitroblue tetrazolium was illuminated for 15 min at $350 \mu\text{mol m}^{-2} \text{ s}^{-1}$ light intensity and measured at 560 nm. For measurements of all enzyme activities, the protein concentration was determined at 595 nm according to Bradford (1976).

2.8. Determination of sodium and potassium concentrations

Approximately 300 mg of the finely ground and dried (80 °C) sample was digested with 69 % HNO_3 in a microwave pressure digestion system (Microwave Reaction System, Multiwave 5000, Anton Paar, Graz, Austria) at 180 °C for 30 min, then diluted to a final volume of 50 mL with distilled water. An ICP optical emission spectrometer (Avio 220 Max, PerkinElmer Inc., Rodgau, Germany) was used to analyze sodium (Na) and potassium concentrations (K) in the filtered samples.

2.9. Grain yield and traits related to grain yield

The aboveground biomass weight per plant (BM) was measured after oven drying the samples at 30 °C to reach a constant weight. The spike weight per plant (Spwt) and the grain weight per spike (GW) were measured, and the number of grains per spike (GN) was counted.

2.10. Data analysis

Analysis of variance (ANOVA) was performed using a mixed linear model with replications as a random effect and treatment and genotype as fixed effects in the “nlme” package with R version 4.3.0 (www.r-project.org). Pairwise comparisons were performed using the “emmeans” function and the same model to test for differences between two species within

each treatment level. Post-hoc pairwise comparisons were conducted using Tukey's test, and the compact letter display (cld) function was applied to provide a visual representation of significant differences between the treatment by genotype combinations. Factoextra (<https://cran.r-project.org/web/packages/factoextra/index.html>) and FactoMineR (<https://cran.r-project.org/web/packages/FactoMineR/index.html>) were used for principal component analysis (PCA). The correlation plots were generated using the corrplot package in R (<https://cran.r-project.org/web/packages/corrplot/index.html>). Figures were created using ggplot2 (<https://cran.r-project.org/web/packages/ggplot2/index.html>) and Microsoft Excel.

3. Results

3.1. Leaf water status

Drought stress adversely affected plants' water status, as evidenced by significant treatment effects on WP, OP, RWC, and AWC (Fig. 1A–1D), when averaged across all genotypes. Among these traits, only WP showed a significant genotype effects and a significant treatment × genotype interaction. Among all the genotypes, *T. araraticum* (G205) showed the greatest reduction in leaf water status under drought stress. At the species level, WP, OP, and RWC were significantly lower in *T. araraticum* than in *T. aestivum* under drought stress conditions, but no such significant differences under well-watered conditions.

3.2. Lipid peroxidation

The MDA content showed significant treatment and genotype effects, but no significant treatment × genotype interaction effect were seen when averaged across all genotypes (Fig. 1E). Under both well-watered and drought stress treatments, *T. araraticum* exhibited significantly lower MDA content for each treatment compared with *T. aestivum* at the species level. Notably, none of the individual genotypes showed a significant treatment effect for MDA content.

3.3. Organic solutes and ion accumulation

The treatment effect, averaged across all genotypes, was highly significant for both proline content (Fig. 1F) and TSS content (Fig. 1G). However, genotype and treatment × genotype interaction effects were not significant for either trait. Under the well-watered treatment, no significant differences were observed between the modern cultivars of *T. aestivum* and *T. araraticum* at the species level or among individual genotypes. However, under drought stress, *T. araraticum* accumulated significantly more proline content than *T. aestivum*, averaged at the species level. Notably, proline accumulation was significantly higher in *T. araraticum* (G205) than in Nordkap under drought conditions. A significant increase in TSS

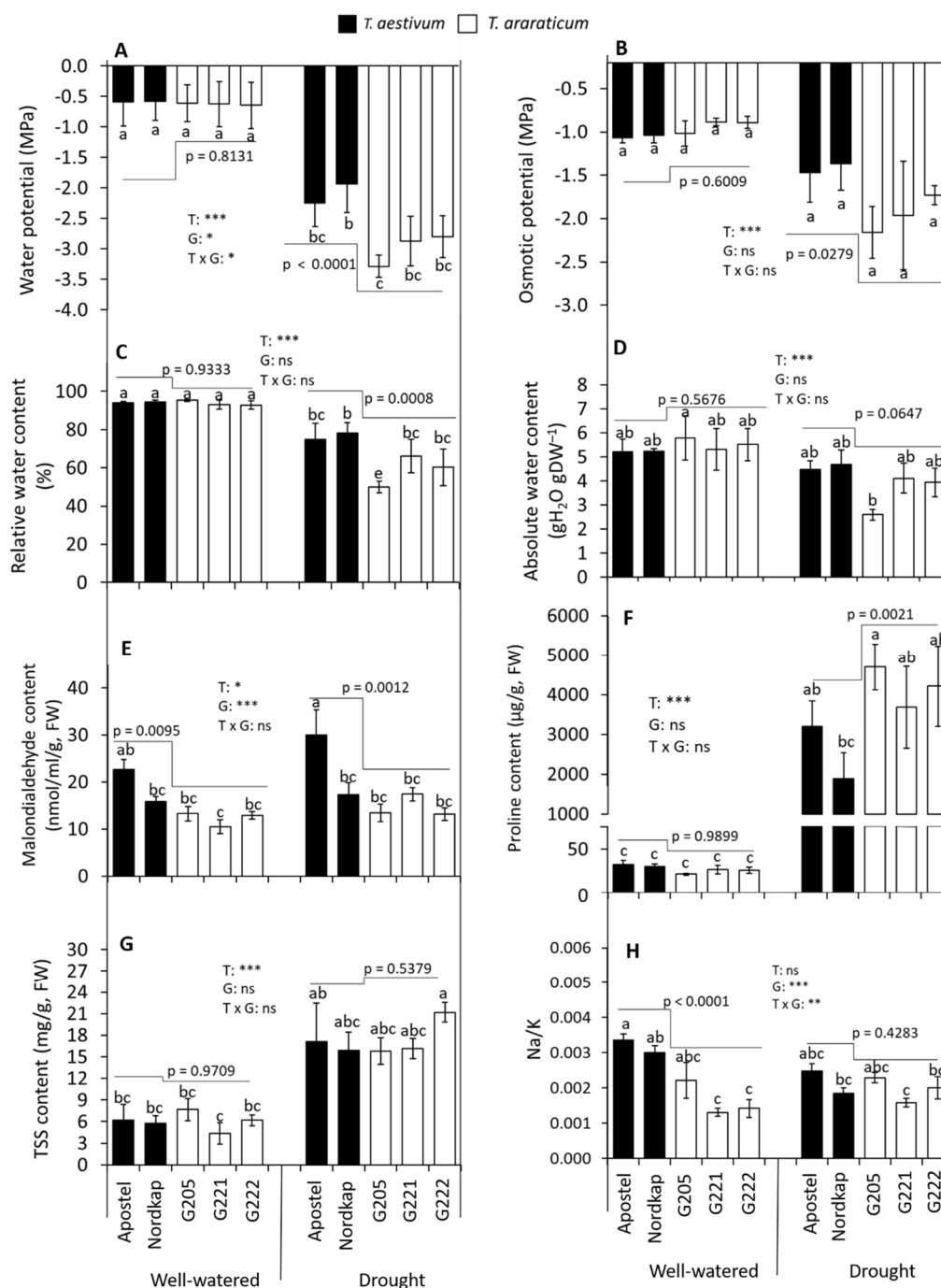


Fig.1: Physiological and biochemical traits measured in leaves of different *Triticum* genotypes grown under well-watered and drought conditions: water potential (A), osmotic potential (B), relative water content (C), absolute water content (D), malondialdehyde content (E), proline content (F), TSS = total soluble sugars content (G), and Na/K ratio (H). Vertical lines on the bars indicate standard errors (n = 4). The letters above the bars indicate significant differences across treatment × genotype interactions determined through pairwise comparisons using Tukey's test. The black and white bars respectively represent modern cultivars of *T. aestivum* and *T. araraticum* genotypes. The p-value shown by the elbow connector above the bars represents the comparison of the mean between two species within the same treatment. Treatment (T), genotype (G), and treatment × genotype interaction (T × G) effects were tested with a two-way ANOVA. The ANOVA significance levels were: *p < 0.05; **p < 0.01; *** p < 0.001; ns = not significant.

accumulation was only observed in *T. araraticum* (G222) under drought stress.

The Na/K ratio showed highly significant effects for genotype and treatment × genotype interactions, but no significant treatment effect was observed (Fig. 1H). Drought stress significantly increased K concentration but had no effect on Na concentration, averaged across all genotypes (Fig. S2A–S2B).

3.4. Leaf gas exchange and water use efficiency

Drought stress significantly reduced C_i , E , g_{sw} , and Φ_iPS2 , whereas it significantly increased WUE_{leaf} but had no significant treatment effect on P_n (Fig. 2A–2F), when averaged across all genotypes. Furthermore, at the species level, leaf gas exchange parameters, such as P_n (Fig. 2A) and WUE_{leaf} (Fig. 2E), were significantly higher for *T. aestivum* under the well-watered treatment compared with *T. araraticum*. However, under the drought stress treatment, there were no significant differences between modern cultivars of *T. aestivum* and *T. araraticum*. All genotypes showed a significant treatment effect for g_{sw} except Nordkap. Moreover, the genotype effect was significant for g_{sw} (Fig. 2D), SD, and SZ (Fig. 2G–2H).

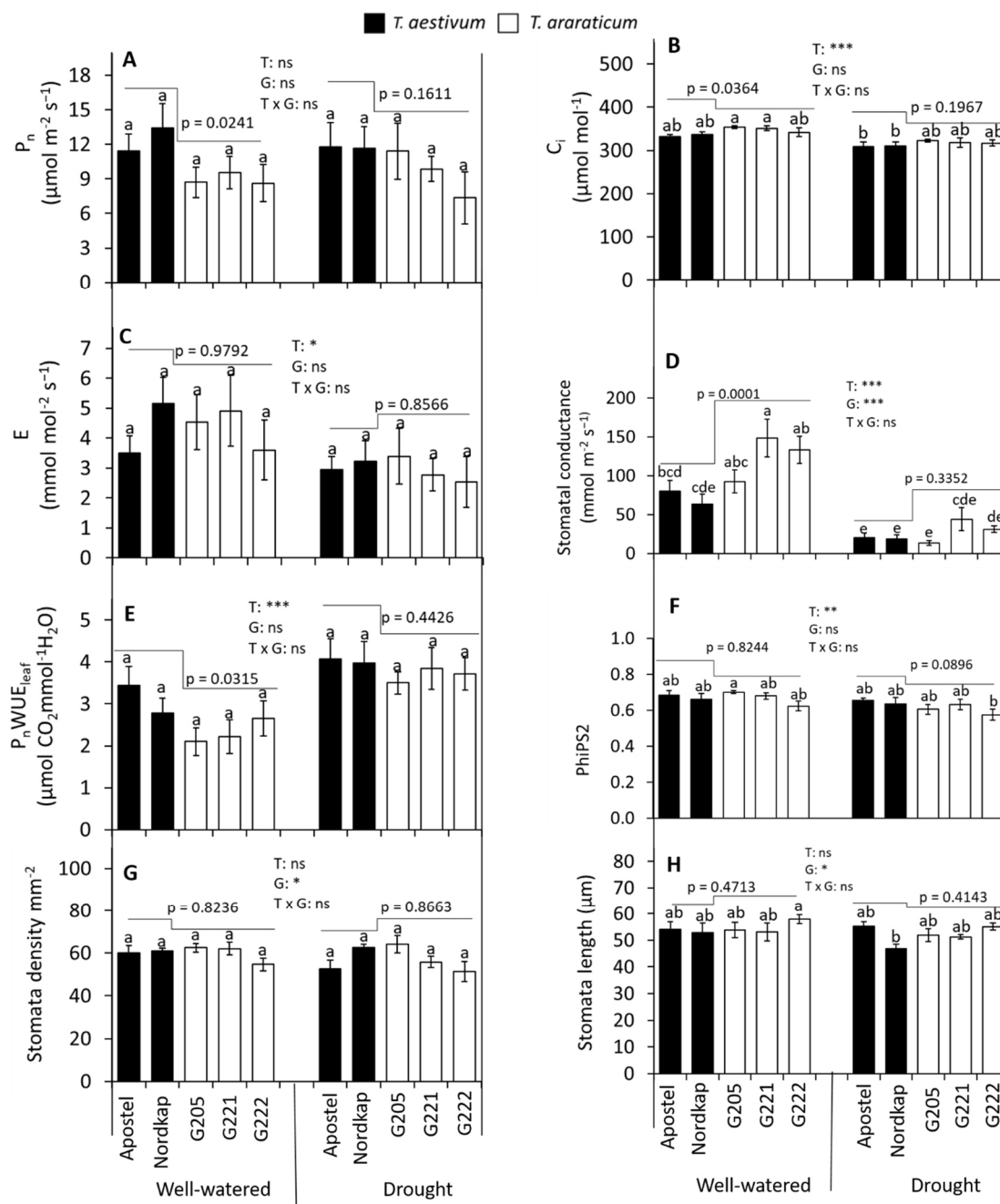


Fig. 2: Leaf gas exchange parameters of different *Triticum* genotypes grown under well-watered and drought conditions: P_n = net photosynthetic rate (A), C_i = internal CO_2 concentration (B), E = transpiration rate (C), stomatal conductance (D), P_nWUE_{leaf} = instantaneous photosynthetic water use efficiency (E), Φ_{iPS2} = quantum efficiency of photosystem II (F), stomata density (G), and stomata length (H). Vertical lines on the bars indicate standard errors ($n = 4$), and for stomatal conductance and quantum efficiency of photosystem II $n = 6$. The letters above the bars indicate significant differences across treatment by genotype interactions determined through pairwise comparisons using Tukey's test. The black and white bars respectively represent modern cultivars of *T. aestivum* and *T. araraticum* genotypes. The p-value shown by the elbow connector above the bars represents a comparison of the mean between two species within the same treatment. Treatment (T), genotype (G), and treatment \times genotype interaction (T \times G) effects were tested with a two-way ANOVA. ANOVA significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant.

3.5. SPAD value

SPAD value was significantly increased due to drought stress, averaged across all genotypes (Fig. S2C). However, individual genotypes did not show significant treatment effects relating to the SPAD value.

3.6. Non-enzymatic antioxidants

The genotype effect was highly significant for total AsA (Fig. 3A), reduced AsA (Fig. S3A), and redox states AsA (Fig. 3B). There was a significant treatment effect for various non-enzymatic antioxidants, such as total GSH (Fig. 3C) and GSH (Fig. S3B) as well as the redox state of both AsA and GSH (Fig. 3B and 3D). Total GSH and GSH as well as the redox state of GSH were enhanced due to drought stress. Conversely, the redox state of AsA was reduced due to drought stress. The treatment × genotype interaction effect was significant for total AsA, redox state AsA, and redox state GSH.

Total AsA and reduced AsA were significantly higher in *T. aestivum* than in *T. araraticum* only under the well-watered treatment (Figs. 3A and S3A) at the species level. However, redox state AsA was significantly higher in *T. aestivum* than in *T. araraticum* only under drought stress across species. *T. araraticum* (G205 and G221) significantly reduced the redox state AsA due to drought stress. Similarly, GSH content was significantly higher in *T. aestivum* than in *T. araraticum* only under the well-watered condition.

3.7. Enzymatic antioxidants

Our analysis extended to the activity of enzymatic antioxidants involved in the AsA–GSH cycle. There was a significant treatment effect for most enzymes apart from APX and AO, such as SOD, POD, DHAR, MDHAR, and GR (Fig. 3E–3J and Fig. S3C). The genotype effect was also highly significant for various enzyme activities, such as SOD, POD, DHAR, GR, and AO. However, only MDHAR activity revealed a significant treatment × genotype interaction effect. MDHAR and GR activity were significantly enhanced in *T. araraticum* (G205 and G222) and *T. araraticum* (G222) respectively.

At the species level, MDHAR activity was significantly higher in modern cultivars of *T. aestivum* than in *T. araraticum* only under the well-watered treatment. However, GR activity was significantly higher in *T. araraticum* than in modern cultivars of *T. aestivum* only under the drought treatment (Fig. 3I). SOD activity was significantly higher in *T. araraticum* than in *T. aestivum* (Fig. 3E) while DHAR and POD (Figs. 3H, 3J) were significantly lower in *T. araraticum*, under both treatments at the species level. DHAR activity was significantly enhanced only in *T. araraticum* (G221) due to drought stress. There was no significant response to drought stress for AO activity in individual genotypes (Fig. S3C).

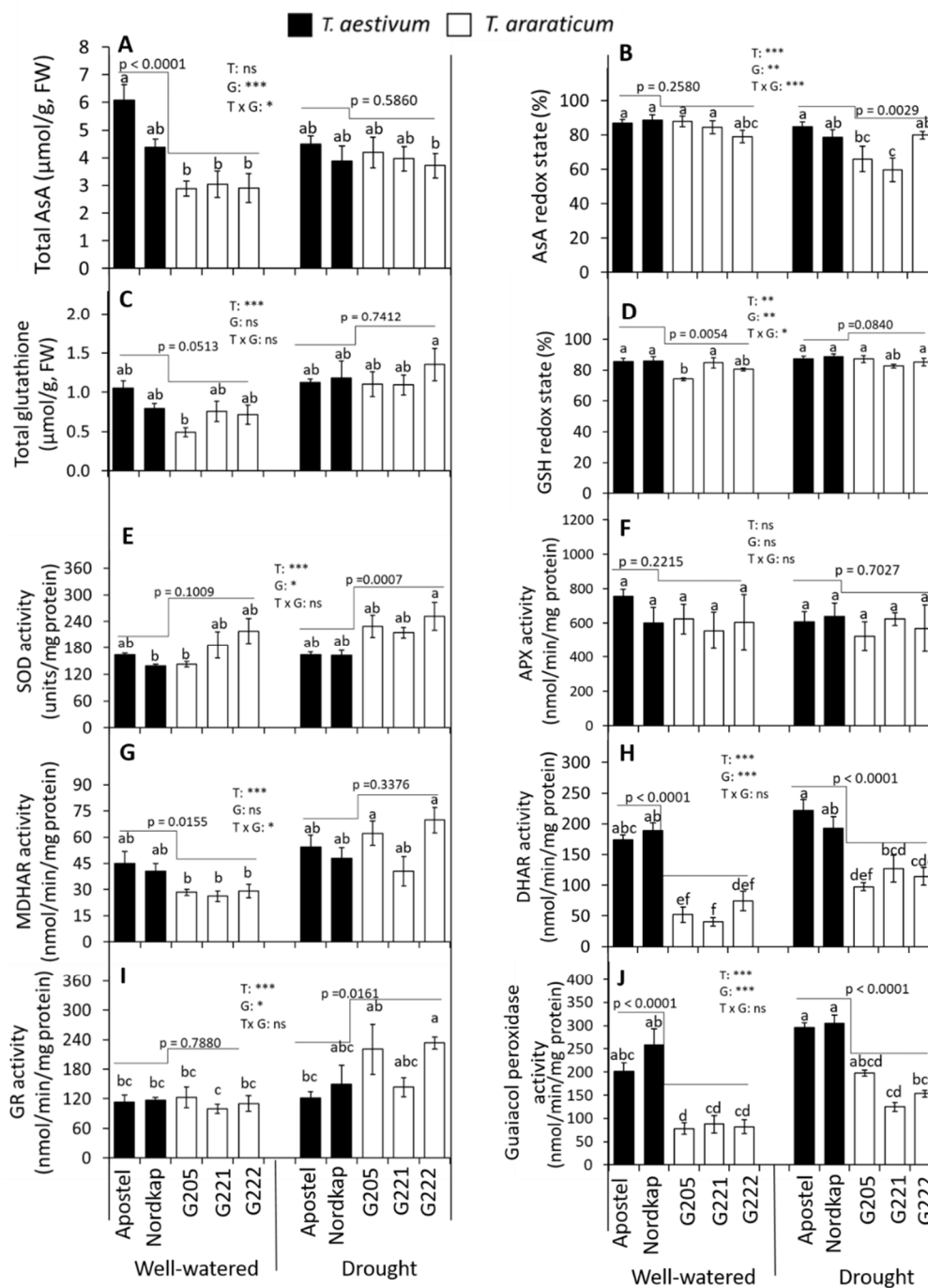


Fig. 3: Antioxidants in leaves of different *Triticum* genotypes grown under well-watered and drought conditions: total AsA = total ascorbate (A), AsA redox state (B), total glutathione (C), GSH redox state = glutathione redox state (D), SOD = superoxide dismutase (E), APX = ascorbate peroxidase (F), MDHAR = monodehydroascorbate reductase (G), DHAR = dehydroascorbate reductase (H), GR = glutathione reductase (I), and guaiacol peroxidase (J). Vertical lines on the bars indicate standard errors (n = 4). Letters above the bars indicate significant differences across treatment by genotype interactions determined through pairwise comparisons using Tukey's test. The black and white bars respectively represent modern cultivars of *T. aestivum* and *T. araraticum* genotypes. The p-value shown by the elbow connector above the bars represents a comparison of the mean between two species within the same treatment. Treatment (T), genotype (G), and treatment × genotype interaction (T × G) effects were tested with a two-way ANOVA. ANOVA significance levels: * p < 0.05; ** p < 0.01; *** p < 0.001; ns = not significant.

3.8. Aboveground biomass and grain yield

Aboveground biomass per plant (BM), Spwt per plant, and GW and GN per spike, averaged across all genotypes, were significant for treatment and genotype effects but not for the treatment × genotype interaction effect (Fig. 4A–4D). BM per plant and Spwt per plant, averaged across the species within the treatment, were significantly higher in *T. aestivum* than in *T. araraticum* only under the drought treatment. Moreover, *T. aestivum* showed significantly higher GW and GN, averaged across species under both treatments than did *T. araraticum*.

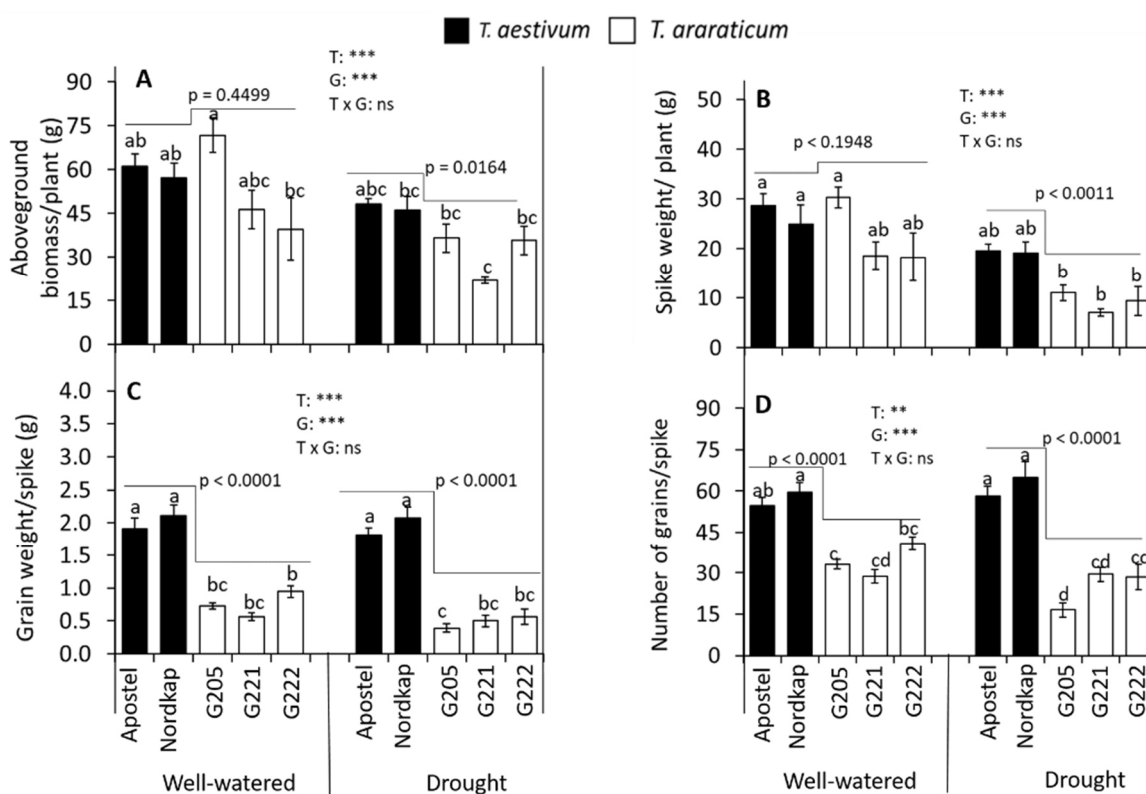


Fig. 4: Yield-related traits of different *Triticum* genotypes grown under well-watered and drought conditions: aboveground biomass per plant (A), spike weight per plant (B), grain weight per spike (C), and number of grains per spike (D). Vertical lines on the bars indicate standard errors (n = 4). Letters above the bars indicates significant differences across treatment by genotype interaction determined through pairwise comparisons using Tukey's test. The black and white bars respectively represent modern cultivars of *T. aestivum* and *T. araraticum* genotypes. The p-value shown by the elbow connector above the bars represents the comparison of the mean between two species within the same treatment. Treatment (T), genotype (G), and treatment × genotype interaction (T × G) effects were tested with a two-way ANOVA. ANOVA significance levels: ** p < 0.01; *** p < 0.001; ns = not significant.

3.9. Associations of physiological and biochemical parameters

The associations between the measured physiological and biochemical traits were analyzed using the PCA biplot (Fig. 5) and correlation plots (Figs. S4 and S5). PC1 revealed

61.7 % variations in *T. araraticum* and 58.9 % in *T. aestivum*. The PCA plots showed clusters in both *T. araraticum* (Fig. 5A) and *T. aestivum* (Fig. 5B) differentiated by treatment. Drought stress was to some extent related to WUE_{leaf} , proline content, TSS content, and some enzymatic antioxidants like MDHAR, GR, POD, and GSH. Proline and TSS contents were negatively correlated with WP.

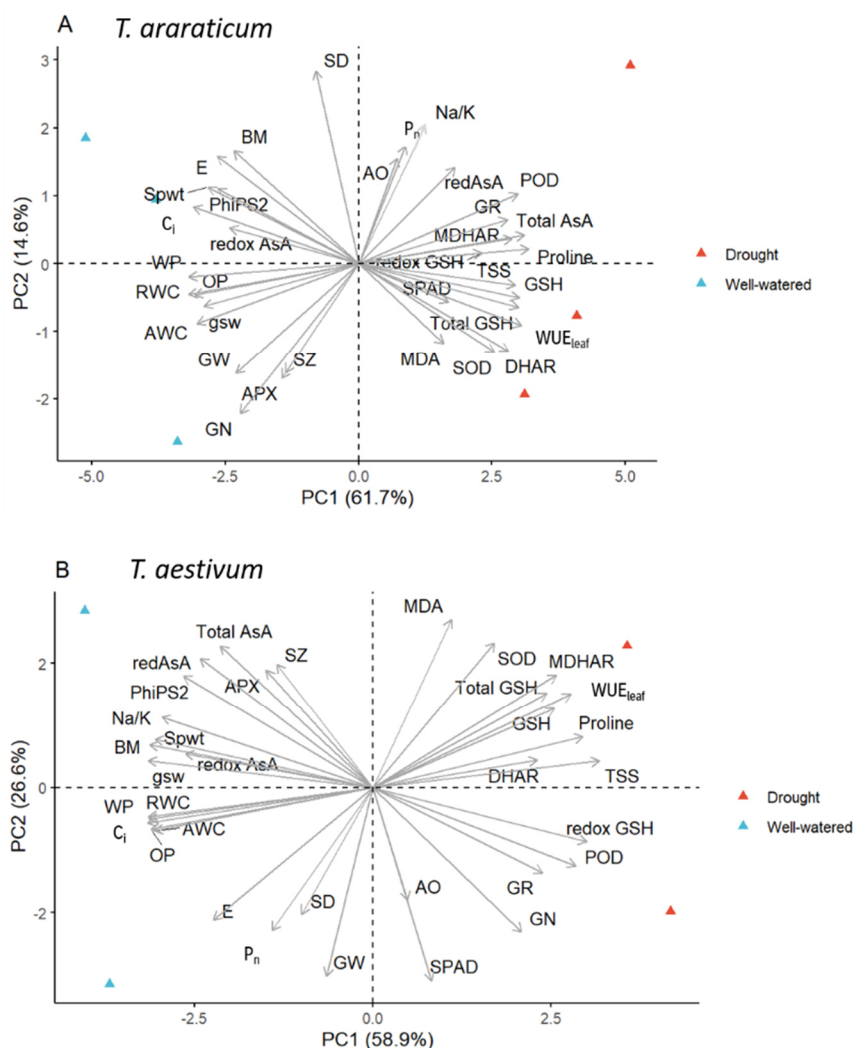


Fig. 5: Principal component analysis (PCA) biplot showing the distributions of the measured traits in *T. araraticum* (A) and *T. aestivum* (B). AO = ascorbate oxidase, APX = ascorbate peroxidase, AsA = ascorbate, AWC = absolute water content, BM = above-ground biomass per plant, C_i = internal CO_2 concentration, DHAR = dehydroascorbate reductase, E = transpiration rate, GN = number of grains per spike, gsw = stomatal conductance, GR = glutathione reductase, GSH = glutathione, GW = grain weight per spike, MDA = malondialdehyde, MDHAR = monodehydroascorbate reductase, Na/K = ratio of sodium to potassium concentration, OP = osmotic potential, PhiPS2 = photosystem II efficiency, P_n = net photosynthetic rate, POD = guaiacol peroxidase, redAsA = reduced ascorbate, RWC = relative water content, SD = stomata density, SOD = superoxide dismutase, Spwt = spike weight per plant, SZ = stomata length, TSS = total soluble sugar content, WP = leaf water potential, WUE_{leaf} = instantaneous photosynthetic water use efficiency.

4. Discussion

Our first hypothesis was that high-yielding modern bread wheat cultivars would exhibit a higher photosynthetic rate with improved gas exchange traits and higher antioxidant levels under well-watered conditions compared with *T. araraticum*. To test this, we compared these traits between modern *T. aestivum* cultivars and *T. araraticum* under well-watered conditions. We found that key gas exchange traits and the levels of most antioxidants were higher in *T. aestivum* compared with *T. araraticum*, with a few exceptions, thereby partially supporting our first hypothesis.

Modern *T. aestivum* exhibited higher P_n and WUE_{leaf} under well-watered conditions (Fig. 2A and 2E), reflecting breeding efforts to enhance resource use efficiency, when resources are not limiting factors. This likely contributes to grain yield (Reynolds et al., 2011), as evidenced by the significantly higher GW and GN in *T. aestivum* compared with those in *T. araraticum* (Fig. 4C–4D). Antioxidants like AsA, GSH, MDHAR, DHAR, and POD enzymes (Figs. 3A, 3G–3J and S3A–S3B) were also higher in *T. aestivum*, likely due to unintentional selection during the breeding process. Our results align with Mao et al. (2018), showing higher photosynthetic and antioxidant activity in modern wheat cultivars. Notably, the modern cultivar Apostel had significantly higher total and reduced AsA content than all three *T. araraticum* genotypes. Therefore, our findings suggest that even though they were not directly targeted, physiological or biochemical traits were unintentionally improved in modern cultivars as a consequence of breeding process aimed at increasing yield (Brestic et al., 2018).

Contrary to previous studies (Khazaei et al., 2010; Wall et al., 2023), we found no significant variation in stomata density and length between *T. aestivum* and *T. araraticum*. However, *T. araraticum* showed higher g_{sw} under well-watered conditions, suggesting a role for stomata aperture rather than density or length. This study is the first to report stomata density and length in *T. araraticum*, highlighting the need for further investigation into the complex functions of stomata in drought tolerance, as stomatal conductance depends on complex functions of stomata (Franks et al., 2009).

Our second hypothesis was that *T. araraticum* exhibits a stronger drought response, with enhanced osmotic adjustments and higher antioxidant activity compared with modern wheat cultivars of *T. aestivum*, thereby enhancing its photosynthetic capacity under drought stress. To test this, we examined the effect of drought stress on various physiological and biochemical traits in both species. *Triticum araraticum* exhibited a stronger drought response with enhanced osmotic regulators, such as proline and TSS accumulation,

increased SOD and GR activities, and maintained photosynthetic capacity under drought stress, partially supporting for our hypothesis.

Drought significantly affected stomatal conductance (Fig. 2D), likely to conserve water through stomatal closure (Farooq et al 2024; Li et al., 2017). However, the increasing trend in WUE_{leaf} in response to drought stress aligned with the finding of Ru et al. (2024), likely to balance the trade-off between CO_2 uptake for photosynthesis and water loss through transpiration. This is crucial for drought tolerance and crop productivity (Akula et al., 2024; Blum, 2009). Notably, *T. araraticum* genotypes (G205 and G222) showed the highest response for WUE_{leaf} . Under well-watered conditions, *T. aestivum* had higher P_n and WUE_{leaf} than *T. araraticum*, but under drought stress, there was no significant difference in gas exchange parameters between the two species. This suggests that *T. araraticum* may maintain photosynthetic rate and water use efficiency better under drought stress, further supporting our second hypothesis.

Our findings showed that drought stress significantly enhanced proline and TSS content in wheat (Fig. 1F–1G), aligning with previous reports (Ru et al., 2023; Saghour el idrissi et al., 2023). A negative association between RWC or water potential and proline and TSS content was also observed (Figs. 5 and S4), consistent with previous studies (Saghour el idrissi et al., 2023; Sallam et al., 2019). Higher proline and TSS accumulation in *T. araraticum* likely contributed to OA and the maintenance of cellular function under drought stress (Sallam et al., 2019; Turner et al., 2007).

Plants' antioxidative mechanisms become increasingly active in response to drought, mitigating the harmful effects of ROS (Naderi et al., 2020; Sallam et al., 2019). Our study showed an increase in most antioxidants under drought stress (Fig. 3A–3J). *T. araraticum* exhibited significantly higher SOD activity compared to *T. aestivum*, supporting previous findings (Suneja et al., 2017). SOD plays a key defensive role against ROS and is an important trait for determining drought tolerance in wheat (Ahmadi et al., 2018; Mehrabad Pour-Benab et al., 2019; Suneja, et al., 2017). MDHAR and GR activities increased significantly in certain genotypes of *T. araraticum* but not in modern bread wheat cultivars, consistent with those of Pour-Aboughadareh et al. (2017) and Suneja et al. (2017), who reported a stronger enzymatic antioxidant response to drought in wild wheats. Furthermore, *T. araraticum* showed a differential reliance on components of the AsA–GSH cycle for mitigating oxidative stress, with a significant increase in GSH redox state, which is an important characteristic, distinguishing them from *T. aestivum*.

POD activity increased in response to drought, consistent with previous findings (Ahmadi et al., 2018; Mehrabad Pour-Benab et al., 2019). However, APX activity showed no response to drought stress. Notably, POD and DHAR activities were significantly greater in

modern varieties of *T. aestivum* than in *T. araraticum* and also MDA levels were higher. These findings of high MDA and POD content in modern wheat are consistent with previous findings (Zhang and Kirkham, 1994).

Notably, none of the above-discussed studies on wild wheats, which focused on the photosynthetic rate, stomatal traits, and antioxidative capacity, included *T. araraticum*. Instead, they investigated other wild wheat species. Therefore, our study provides new insight in this respect. However, it had limitations, such as measuring antioxidants only at the end of the stress period and variations in phenological stages (e.g., earlier heading in *T. araraticum*; Table S1). Phenophase differences between species can influence yield outcomes, making it more complex to compare yield traits between species under drought conditions. These limitations should be addressed in the future. Moreover, future studies should focus on additional parameters like stomatal aperture and dynamic changes in antioxidant responses.

5. Conclusion

While some within-species variation in drought responses was found, our findings showed several consistent patterns of differential drought responses between species. Under non-stress conditions, modern *T. aestivum* cultivars exhibited higher gas exchange and antioxidant activity compared with *T. araraticum*, which could contribute to their higher grain yield. Drought stress significantly enhanced osmoprotectants, like TSS and proline content. Additionally, most of the antioxidants involved in the AsA–GSH cycle were enhanced in response to drought stress. Of the two species, *T. araraticum* showed a stronger response. Our results demonstrated differential antioxidant mechanisms for maintaining the redox balance via the AsA–GSH cycle in two different species. *T. araraticum* may rely more on GSH rather than AsA, with enhanced MDHAR and GR in response to drought. By contrast, modern cultivars of *T. aestivum* showed higher base levels of POD and DHAR activities for maintaining the redox state of AsA. The specific drought adaptation mechanism of *T. araraticum* could prove useful in certain drought scenarios and could be harnessed by using the species as breeding resources or via *de novo* domestication.

Author contributions

Sumitra Pantha: conceptualization, methodology, investigation, formal analysis, visualization, and writing - original draft; **Benjamin Kilian**: writing - review & editing; **Hakan Özkan**: writing - review & editing; **Muhammad Farooq**: writing - review & editing; **Frederike Zeibig**: writing - review & editing; and **Michael Frei**: conceptualization, resources, writing - review & editing.

Funding statement

This work was supported by the German Academic Exchange Service (DAAD) under the Programm des Projektbezogenen Personenaustauschs (PPP) Project [project number 57627943] and the Biodiversity for Opportunities, Livelihoods and Development (BOLD) Project funded by the Government of Norway [grant number QZA-20/0154]. SP received a PhD scholarship from DAAD. The funders did not have any role in the study design or implementation.

Acknowledgments

The authors are grateful to Liane Renno, Erika Schick, and Moritz Jehner for their assistance during the greenhouse experiment and laboratory work and for their help in threshing, cleaning, and measuring the samples. We would also like to thank Plant Nutrition Department of the University of Giessen for providing the facility for measuring osmotic potential. ki@JLU was used to improve the legibility.

Data availability:

All data relevant to this article can be found in the article and in the supplementary material.

Declarations:

Conflicts of interest: The authors declare no competing interests.

Code availability: Not applicable

Abbreviations:

AO = ascorbate oxidase, APX = ascorbate peroxidase, AsA = ascorbate, AWC = absolute water content, BM = above-ground biomass per plant, C_i = internal CO₂ concentration, DHAR = dehydroascorbate reductase, DTNB = 5-dithiobis-(2-nitrobenzoic acid), DTT = dithiothreitol, DW = dry weight, E = transpiration rate, FW = fresh weight, GN = number of grains per spike, GR = glutathione reductase, GSH = glutathione, GSSG = oxidized glutathione, gsw = stomatal conductance, GW = grain weight per spike, MDA = malondialdehyde, MDHAR = monodehydroascorbate reductase, NADPH = nicotinamide adenine dinucleotide phosphate, Na/K = ratio of sodium to potassium concentration, OA = osmotic adjustment, OP = osmotic potential- ψ_s , PCA = principal component analysis, PhiPS2 = photosystem II efficiency, P_n = net photosynthetic rate, POD = guaiacol peroxidase, PPFD = photosynthetically active photon flux density, PVPP = polyvinylpyrrolidone, PWHC = pot soil water-holding capacity, ROS = reactive oxygen species, redAsA = reduced ascorbate, RWC = relative water content, SD = stomata density,

SOD = superoxide dismutase, Spwt = spike weight per plant, SZ = stomata length, TCA = trichloroacetic acid, TW = turgid weight, TSS = total soluble sugar content, WP = leaf water potential- ψ_w , WUE_{leaf} = instantaneous photosynthetic water use efficiency.

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5 General discussion

5.1 Outline

Wheat is a vital crop for global food security, with demand expected to rise alongside population growth (Feldman & Levy, 2023; Langridge et al., 2022). However, its productivity is decreasing due to environmental stresses such as drought (Farooq et al., 2014; Khadka et al., 2020). Wild wheat relatives, originating from the Fertile Crescent, are invaluable for their adaptation to harsh climates, including arid regions with hot, dry summers (Dempewolf et al., 2017; Jaradat, 2017; Zeibig et al., 2024a). They provide crucial resources for crop scientists aiming to enhance drought tolerance in cultivated wheat (Nevo & Chen, 2010). To identify beneficial drought-adaptive traits, screening the extensive gene pool of wheat under relevant stress conditions is essential (Aberkane et al., 2021). Previous studies have investigated these mechanisms in wild wheat relatives (Peleg et al., 2009; Suneja et al., 2017, 2019), but comprehensive research examining the diverse gene pool of *Triticum*, with detailed physiological and morphological traits under both field and controlled conditions, remains limited.

The objective of this thesis was to explore drought-adaptive traits in the *Triticum* spp. and their underlying tolerance mechanisms by examining agromorphological and physiological responses to drought stress. Wild wheat relatives, landraces, and modern cultivars were evaluated for various agromorphological and physiological parameters through field and greenhouse experiments under water stress (rainfed or drought) and optimal (irrigated or well-watered) conditions. This general discussion aims to summarize and compare the findings presented in Chapters 2, 3, and 4.

5.2 A comparison of greenhouse and field experiments

In this study, a field screening experiment was conducted under rainfed and irrigated conditions using a large gene pool of *Triticum*. Subsequently, the selected genotypes were evaluated for their drought responses based on morphological and physiological traits over two seasons, presented in Chapter 2. These selected genotypes were further tested in a greenhouse experiment under well-watered and drought stress conditions. Stress was applied first during the stem elongation stage and then during the anthesis stage, described in Chapter 3. Therefore, it is worth comparing the results of the field experiment with those of the greenhouse experiment, focusing on important traits such as stomatal conductance and grain yield, to see how responses to water stress differ among genotypes in these two experiments.

5.2.1 Stomatal conductance

The main conclusions drawn regarding stomatal regulation from both the field experiment (Chapter 2) and the greenhouse experiment (Chapter 3) were:

- ❖ Water stress significantly affects stomatal conductance
- ❖ Genotypes differentially regulate their stomatal responses to water stress

Specifically, certain genotypes of wild wheats such as *T. dicoccoides* (G242), *T. urartu* (G45) and *T. boeoticum* (G27 and G33) showed high response to water stress with isohydric (water-saving) stomatal regulation in the field experiment (Chapter 2). These results could be further confirmed by the greenhouse experiment (Chapter 3), as these genotypes showed a similar response to drought stress in both experiments. Here, stomatal conductance results from the field experiment were compared with those from the greenhouse experiment during the anthesis stage. Table 1 presents a comparison for 17 genotypes included in both the field and the greenhouse experiments. This comparison revealed an interesting finding: the genotypes showing significant differences in stomatal conductance in the field also exhibited these differences in the greenhouse, with a few exceptions, indicating consistent results between the two experiments.

Overall, a broader range of stomatal conductance was observed under greenhouse experiment (approximately 60–785 mmol m⁻² s⁻¹), with a higher maximum value compared to field experiment (approximately 63–153 mmol m⁻² s⁻¹), under optimal conditions (irrigated/well-watered) (Table 1). Stomatal conductance was generally higher in the greenhouse experiment than in the field experiment under optimal conditions at the individual genotype level, with a few exceptions. This result aligns with previous findings (Sales et al., 2022), which reported higher stomatal conductance in greenhouse-grown plants compared to those grown in field conditions. High stomatal conductance observed in greenhouse environments is likely due to the optimized growth conditions, such as light, humidity, and temperature, provided by the controlled experimental chambers in the greenhouse. However, in natural field conditions, these factors may not be stable and can be influenced by wind or variations in air and soil temperatures, which can affect stomatal regulation in plants (Buckley, 2019; Shapira et al., 2024). Controlled greenhouse chambers usually allow for precise control of growth conditions, potentially optimizing physiological functions (Poorter et al., 2016).

In summary, the fundamental stomatal regulation in response to drought stress was largely consistent between the greenhouse and field experiments, although stomatal conductance levels slightly differed under optimal conditions between the two settings. This indicates that the greenhouse experiment largely reflects the field results.

Table 1: Comparison of stomatal conductance from field experiment (2022 and 2023 average) and greenhouse experiment (2023) for 17 genotypes.

		Stomatal conductance, mmol m ⁻² s ⁻¹					
Taxa	Genotype identifier	Greenhouse			Field		
		Well-watered	Drought	ANOVA result	Irrigated	Rainfed	ANOVA result
<i>T. aestivum</i>	G10	231.8	37.0	ns	145.1	57.3	ns
<i>T. boeoticum</i>	G27	681.7	58.6	***	126.2	59.6	*
<i>T. boeoticum</i>	G33	784.5	0.01	***	135.5	47.7	**
<i>T. durum</i> cv. Sambadur	G41	145.3	23.5	ns	115.8	78.8	ns
<i>T. urartu</i>	G45	334.1	48.2	*	153.2	40.6	***
<i>T. monococcum</i> sinskajae	G89	70.3	15.3	ns	103.6	70.2	ns
<i>T. dicoccoides</i>	G90	138.9	19.4	ns	95.7	51.9	ns
<i>T. spelta</i>	G109	60.1	0.2	ns	74.6	66.1	ns
<i>T. aestivum</i> cv. Apostel	G134	80.1	0.2	ns	96.4	74.8	ns
<i>T. araraticum</i>	G161	151.0	104.9	ns	76.7	42.3	ns
<i>T. aestivum</i> cv. Nordkap	G181	190.5	13.5	ns	92.1	67.4	ns
<i>T. araraticum</i>	G189	174.1	119.7	ns	96.5	69.2	ns
<i>T. araraticum</i>	G205	192.6	88.4	ns	73.4	52.3	ns
<i>T. araraticum</i>	G221	202.6	88.0	ns	86.1	35.2	ns
<i>T. araraticum</i>	G222	213.8	96.1	ns	62.7	21.5	ns
<i>T. dicoccoides</i>	G242	289.4	75.7	*	137.4	57.4	***
<i>T. dicoccoides</i>	G248	222.9	105.2	ns	142.0	57.3	***
Maximum		784.5	119.7		153.2	78.8	
Minimum		60.1	0.01		62.7	21.5	
Average		244.9	52.6		106.7	55.8	

Field experiment data were adopted from Chapter 2, and greenhouse experiment data (at anthesis stage) were adopted from Chapter 3. The mean comparison was done using two-way ANOVA with different models for greenhouse (Pantha et al., 2024) and field condition (Pantha et al., 2025). Data from the greenhouse and field experiments were analyzed separately. *, **, and *** denote significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns = nonsignificant.

5.2.2 Yield-related traits

A correlation analysis between the results from field and greenhouse experiments for yield-related traits revealed a positive association (Figure 2A–2C), suggesting that greenhouse experiment results reflect those observed in the field. Positive correlations were observed for key yield-related traits such as grain yield per plant (Figure 2A), harvest index (Figure 2B), and thousand kernel weight (Figure 2C) between greenhouse and field experiments. Consistent with these findings, Sales et al. (2022) reported that agronomic traits, including grain yield, measured in glasshouse-grown plants show good consistency with those measured in the field.

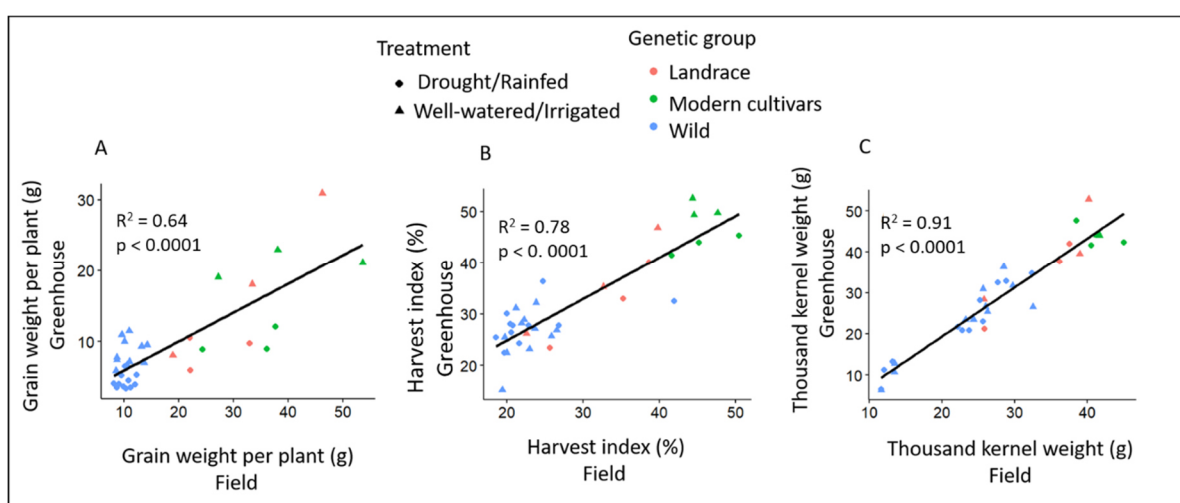


Figure 2: Relationships between field and greenhouse experiments for yield-related traits. Grain weight per plant (A), harvest index (B), and thousand kernel weight (C). Blue, red, and green color denote genetic groups; wild, landrace and modern cultivars, respectively. A triangle shape represents well-watered/irrigated conditions, while a diamond shape indicates drought/rainfed conditions. Data from 17 common genotypes included in both field (Chapter 2) and greenhouse (Chapter 3) experiments were used for the association analysis.

Most individual genotypes exhibited similar response to drought stress in both field and greenhouse experiments, as revealed by the relative value of grain weight (Figure 3A) and harvest index (Figure 3B), with some exceptions. The calculation of relative value and its advantages are described in detail in Chapter 2. The ranking order of the relative values of 17 genotypes (ranked 1 for the highest relative value to 17 for the lowest) was not entirely consistent between the two experiments (Figure 3A and 3B), likely indicating that yield traits are more complex. Therefore, it is necessary to consider the complex interactions among traits that influence crop yield under varying environmental conditions (Sales et al., 2022). Among the genotypes evaluated in both field and greenhouse experiments, a distinct

contrast was observed in the ranking order for the relative grain yield value of *T. durum* cv. Sambadur (G41). It ranked 1st in the field experiment but 11th in the greenhouse experiment (Figure 3A). In the greenhouse experiment (Chapter 3), grain yield per plant for this genotype was significantly decreased due to drought conditions. However, in the field experiment (Chapter 2), the grain yield remained unaffected by water stress, likely because of deep root system of durum wheat cultivar. The roots might have been unrestricted in the field, whereas in the greenhouse they were possibly limited by the confined pot size. Previous findings by El Hassouni et al. (2018) reported that durum wheat accessions achieved high grain yield under water stress conditions due to their deep roots. Although the overall impact of water stress on yield-related traits, such as harvest index and grain yield per plant, followed the same trend in both greenhouse and field experiments, the degree of impact was greater in the greenhouse compared to the field. This difference likely result from the greenhouse experiment conditions, where plants were grown in 5-liter pots under severe drought conditions by withholding irrigation during the stem elongation and anthesis stages. In contrast, the field experiment relied on natural rainfed conditions. To ensure the greenhouse experiment results accurately reflect natural field conditions, it is crucial to consider factors such as pot size and soil moisture status, as highlighted in previous studies by Poorter et al. (2016) and Sales et al. (2022).

In summary, greenhouse experiments are often used to translate field results for agronomic and yield-related traits. However, it is essential to carefully consider experimental conditions, such as space for root growth, soil moisture, and soil type, to ensure that these findings accurately reflect field conditions. This consideration is especially important for complex traits like grain yield. Additionally, drought severity, plant species, and genotypes can also affect the results.

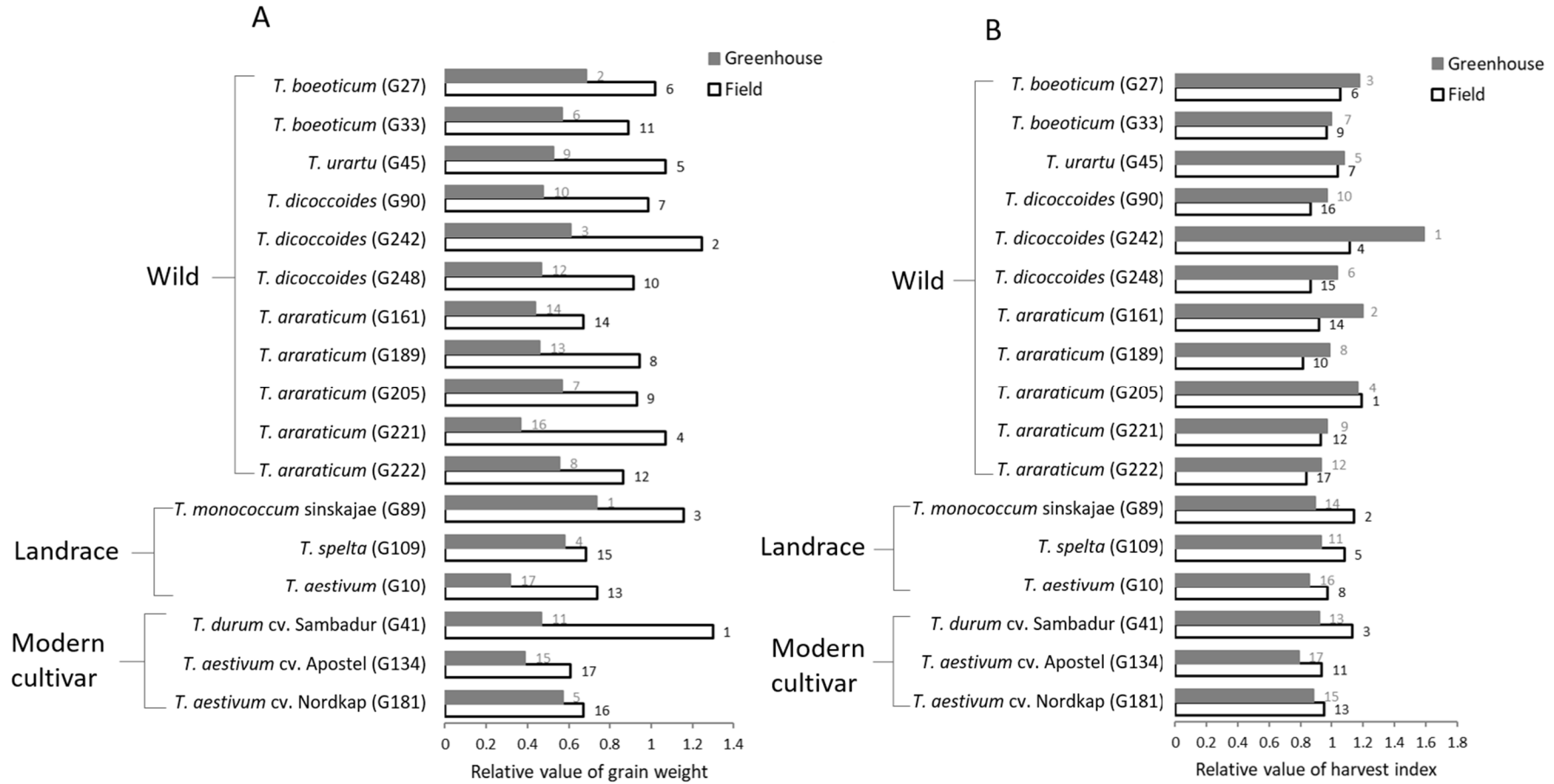


Figure 3: The relative value of yield traits for 17 genotypes included in both field experiment (Chapter 2) and greenhouse experiment (Chapter 3). Relative value of grain weight (A), and relative value of harvest index (B). White bars represent field experiment and grey bars represent greenhouse experiment. The relative values were taken from Chapter 2 for the field experiment and Chapter 3 for the greenhouse experiment. Genotypes were ranked from 1 to 17 (the highest relative value ranked 1 and the lowest relative ranked 17) separately for field and greenhouse experiments. The number on the top of the bars represents the respective ranking order.

5.3 Selection of suitable genotypes for water stress conditions based on relative yield performance and related physiological traits

The *Triticum* spp., consisting of three different genetic groups, were tested for their drought tolerance traits both in the field and under controlled conditions in the greenhouse. Based on their relative yield performance and other physiological traits, drought-tolerant genotypes were selected (Pantha et al., 2024, 25). Wild wheats did not outperform domesticated wheats overall, and both field and greenhouse experiments revealed genotype-dependent response to drought stress, with significant variations in stomatal regulation, yield-related traits, and morphological characteristics, discussed in detail in Chapter 2 and Chapter 3. The performance of individual genotypes varied widely across different traits. Nevertheless, in this dissertation, general conclusions are drawn based on the overall performance of each genotype. To assess the selection of drought-tolerant candidates (Table 2 and Figure 4), the results from separate experiments (Chapters 2, 3 and 4) were considered.

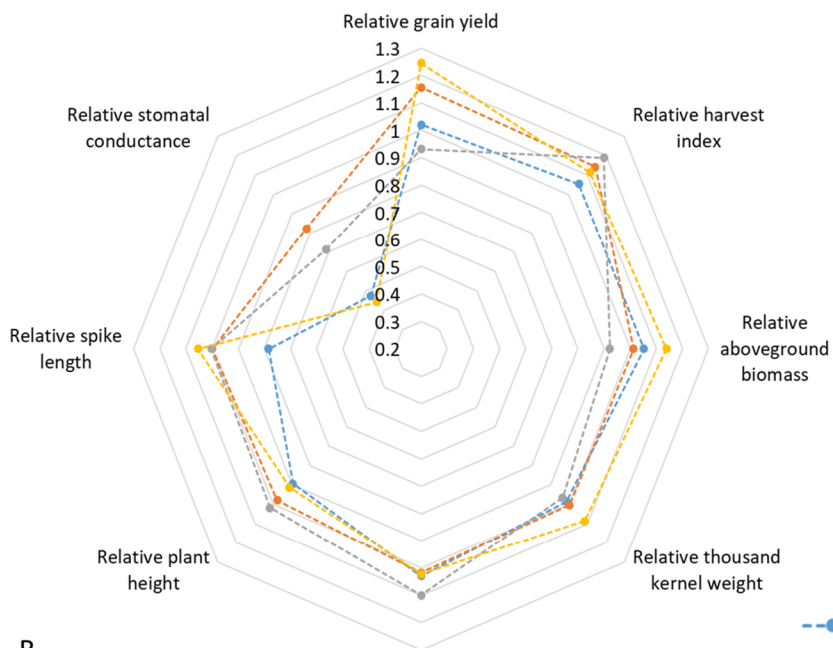
The target drought-tolerant candidate genotypes were selected based on their relative grain yield and responses to water stress in terms of morphological and physiological traits (Table 2 and Figure 4A–4B) (Pantha et al., 2024, 25). *Triticum monococcum* *sinskajae* (G89) and *T. dicoccoides* (G242) performed the best in terms of relative grain yield, followed by *T. boeoticum* (G27) and *T. araraticum* (G205). Therefore, these four genotypes, each belonging to different taxa, were proposed as suitable candidates for drought tolerance. These genotypes maintained grain yield per plant under water stress conditions in both the field (Pantha et al., 2025) and greenhouse experiments (Pantha et al., 2024).

Table 2: List of selected genotypes that performed well under water stress conditions in both greenhouse and field experiments (Pantha et al., 2024, 25).

Genotype identifier	Taxa	Genome	Gene bank identifier	Origin	Drought-adaptive traits
G89	<i>T. monococcum</i> sinskajae	A ^m	ID 69 SSD 2007+2009	Turkey	Reduction in PH, maintainance of RWC (Chapter 3)
G242	<i>T. dicoccoides</i>	BA	KU-14474	Israel	Isohydic stomatal regulation, maintainance of RWC, reduced PH and BM and increased HI (Chapter 3)
G27	<i>T. boeoticum</i>	A ^b	PI 427583	Turkey	Isohydic stomatal regulation, maintainance of RWC (Chapter 3)
G205	<i>T. araraticum</i>	GA ^t	KU-8733	Iraq	Osmotic adjustment (proline accumulation) and enhanced MDHAR activity and balanced redox state GSH (Chapter 3 and Chapter 4)

Abbreviations: BM = aboveground biomass per plant, GSH = glutathione, HI = harvest index, MDHAR = monodehydroascorbate reductase, PH = plant height, and RWC = relative water content.

A



B

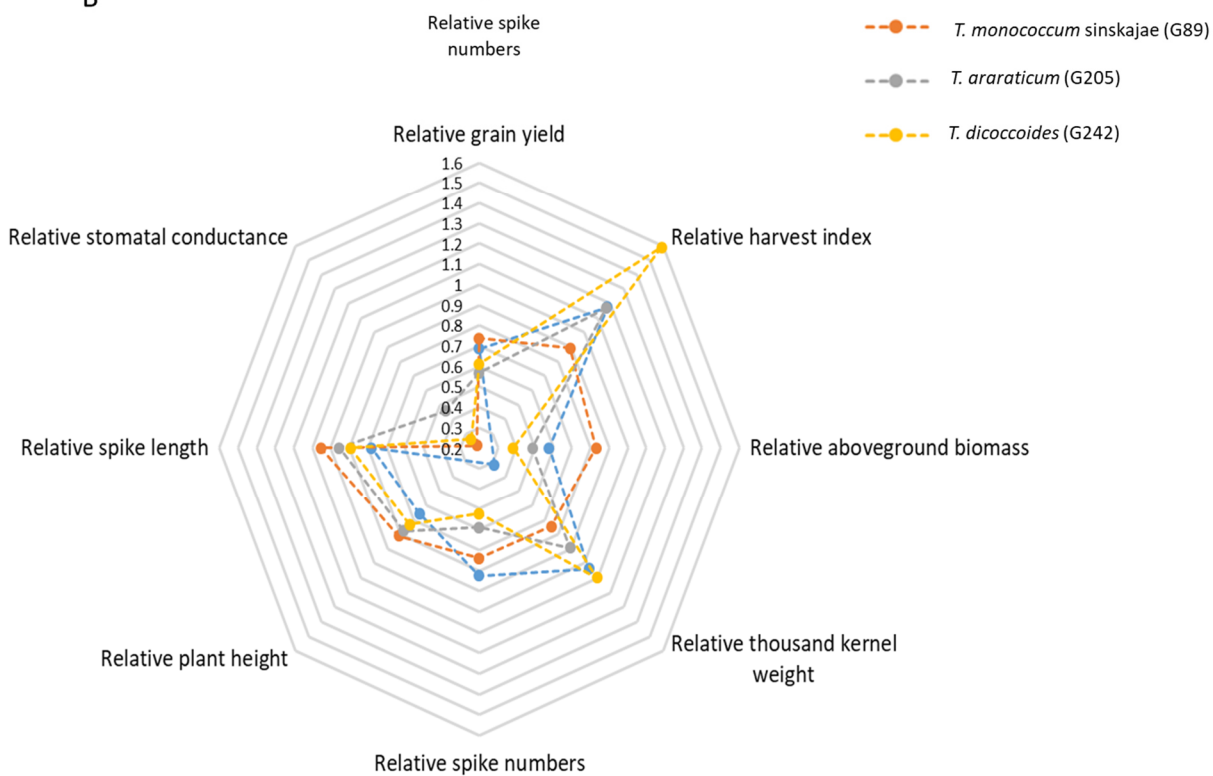


Figure 4: Comparison of the relative value of yield-related traits, morphological traits and stomatal conductance for four selected drought-tolerant genotypes. Field experiment (A), and greenhouse experiment (B). Dotted lines represent the relative value for each genotype.

Triticum monococcum *sinskajae* (G89) exhibited drought adaptation traits such as enhanced anthesis in response to drought, reduction in plant height (PH) and maintenance of relative water content (RWC), while maintaining grain yield per plant (Chapters 2 and 3). Among the four identified drought-tolerant genotypes, *T. dicoccoides* (G242) and *T. boeoticum* (G27) exhibited high response to stomatal conductance, employing isohydric (water-saving) regulation for drought adaptation. This response was consistent across both the field and the greenhouse experiments, while maintaining grain yield per plant. Additionally, genotype *T. dicoccoides* (G242) employed drought escape strategy by enhancing anthesis and exhibited drought tolerance by maintaining RWC. Notably, *T. dicoccoides* (G242) significantly increased the harvest index under severe drought stress in the greenhouse experiment, which could be a valuable trait for breeders aiming to enhance wheat yield potential under water-stress environments. This is likely achieved by drought avoidance strategy of reduced biomass, while maintaining the grain yield per plant, discussed in Chapter 3. Similarly, *T. boeoticum* (G27) showed drought-adaptive traits through enhancing anthesis and maintaining RWC under severe drought stress. Likewise, *T. araraticum* (G205) maintained grain yield per plant and regulated osmotic stress by accumulating proline. Moreover, in a separate experiment evaluating *T. araraticum* genotypes for their antioxidants and gas exchange traits (Chapter 4), the *T. araraticum* (G205) exhibited a strong drought-adaptive response. It also demonstrated enhanced activity of monodehydroascorbate reductase (MDHAR), a balanced redox state of glutathione, and relatively high instantaneous photosynthetic water use efficiency under drought conditions. These drought-tolerant candidates could be incorporated into future wheat improvement programs to enhance drought adaptability.

5.4 Variations of drought tolerance traits in the *Triticum* gene pool

The *Triticum* gene pool exhibits substantial variations for drought tolerance traits, with key findings discussed in Chapters 2, 3, and 4. These traits include stomatal regulation, early anthesis, reduced plant height, decreased aboveground biomass, osmotic adjustment through accumulation of proline and total soluble sugars, enhanced antioxidant activity, and improved water use efficiency in response to drought stress. These findings align with previous studies. For instance, Ru et al. (2023) observed that the accumulation of proline and soluble sugars enhance membrane stability and osmoregulation in plants under drought stress. Similarly, Ahmadi et al. (2018) observed that increased antioxidant activity helps maintain cellular oxidative balance in stressed wheat. Furthermore, the drought escape strategy of early anthesis and maturity has been linked to efficient resource use and high yield potential in crops (Clavel et al., 2005; Regan et al., 1997). The extent of drought-adaptive traits varied significantly among species and genotypes (Pantha et al., 2024, 25),

aligning with previous findings (Pour-Aboughadareh et al., 2017; Suneja et al., 2019; Wang et al., 2017). Wild wheat species such as *T. dicoccoides*, *T. urartu* and *T. boeoticum* adopted an isohydric response, while certain landraces and cultivars such as *Triticum monococcum* *sinskajae* and *T. durum* cv. Sambadur followed an anisohydric response (Pantha et al., 2025). Such variations in stomatal regulations have been reported in previous studies in wheat (Faralli et al., 2024; Gallé et al., 2013; Onyemaobi et al., 2021) and barley (Paul et al., 2024), offering different benefits depending on genotype and drought severity (Bandurska, 2022).

Wild wheat relatives and landraces exhibit considerable variations in agromorphological and physiological traits, as reported by various studies (Pantha et al. 2024, 25; Pour-Aboughadareh et al., 2017; Reynolds et al., 2007; Suneja et al., 2019). These traits include stomatal conductance, photosynthetic capacity, water status, grain yield, and biomass. Similarly, large variations in drought tolerance traits, such as stomatal regulation, photosynthetic activity, and osmotic adjustment, have also been observed in CWRs of other cereals such as rice (Kondamudi et al., 2016), and barley (Cai et al., 2020). Wild wheats and landraces exhibited drought tolerance through mechanisms such as reducing oxidative stress, accumulating high levels of proline, and employing drought avoidance strategies, including reduced stomatal conductance and decreased plant height, presented in Chapters 3 and 4. It was also shown that modern bread wheat cultivars have exhibited drought tolerance primarily by reducing oxidative stress. These findings are consistent with Li et al. (2021), who reported that primitive wheat accessions, including wild and landraces, avoid drought by reducing leaf area and stomatal conductance, whereas modern cultivars maintain a high water potential. Although primitive wheats exhibit reduced tillering and plant height under drought conditions, they prevent total crop failure (Nakhforoosh et al., 2015). In contrast, modern cultivars provide better yields under both optimal and suboptimal conditions (Voss-Fels et al., 2019), but may lead to complete crop failure under severe drought conditions (Nakhforoosh et al., 2015).

Wheat's secondary gene pool, *T. araraticum* (GGAA), has been largely overlooked in studies related to abiotic stress physiology. However, interest in its untapped genetic diversity is growing (Badaeva et al., 2022). *T. araraticum* also exhibited high variations in drought tolerance traits, such as stomatal regulation and proline content, discussed in Chapters 2 and 3. Furthermore, this species exhibited unique drought tolerance characteristics through enhanced antioxidant activities, including superoxide dismutase (SOD), monodehydroascorbate reductase (MDAHR), glutathione reductase (GR), and maintaining glutathione (GSH) redox balance in the ascorbate–glutathione cycle, distinct from modern cultivars, presented in Chapter 4. In conclusion, the *Triticum* genus exhibits

significant genetic variation, with wild wheat relatives and landraces offering unique drought-resilient traits that can enhance the genetic diversity of modern cultivars.

5.5 Trait shifts in crop domestication and breeding for higher yields

As discussed in Chapter 4, domestication and breeding modifications may have indirectly enhanced the photosynthetic rate, water use efficiency, and antioxidant levels in modern cultivars, thereby favoring increased grain yield. This study reported that these physiological traits were more pronounced in modern wheat cultivars than in wild taxa under optimal conditions. These findings align with previous findings (Mao et al., 2018; Wang et al., 2017), which indicated that domestication and breeding have indirectly led to higher photosynthetic rates and biochemical traits in modern cultivars compared to primitive wheat. However, during drought stress, modern cultivars exhibited a greater reduction in photosynthetic rate and a less pronounced increase in antioxidant activity compared to their performance under optimal conditions, whereas wild wheat showed a greater change in these traits, presented in Chapter 4.

Morphological and physiological features have been altered during domestication to enhance crop productivity (Foulkes et al., 2011; Lei et al., 2024). For example, Brestic et al. (2018) demonstrated that modern wheat cultivars exhibit increased photosynthetic rates, which has significantly contributed to improved crop yield. In contrast, studies by Kondamudi et al. (2016) and Mathan et al. (2021) found that wild rice accessions exhibit high photosynthetic rates. Furthermore, domestication effects have been most evident in yield traits, followed by morphological, anatomical, and physiological traits in major crops like soybean, maize, cotton, rice, and wheat (Lei et al., 2024). Chapters 2 and 3 demonstrate that, although modern wheat cultivars have achieved higher yields, they have greater yield penalties due to water stress compared to certain wild wheat genotypes and landraces (Pantha et al., 2024, 25). Yang et al. (2024) also reported that old wheat cultivars perform well even with limited resources, such as low fertilizer and water, whereas new cultivars yield more only when such resources are abundant. In contrast, Voss-Fels et al. (2019) demonstrated that new wheat cultivars can outperform old ones even under low-input conditions, such as when nitrogen and water are scarce. Similarly, Brestic et al. (2018) found that modern wheat cultivars exhibit increased photosynthesis, which boosts yields even under non-optimal conditions like heat stress. This may be due to the unintentional selection of stress-tolerant traits, alongside yield enhancement during rigorous breeding efforts. In summary, while breeding has significantly advanced crop yields by altering physiological and morphological traits, it is important for modern cultivars to balance the trade-offs between resilience and performance under stressful conditions.

5.6 Conclusions and future prospects

This thesis revealed variations in morphological, physiological, and biochemical traits within the *Triticum* gene pool in response to water stress. Wild wheat species were not found to be superior, *per se*, to domesticated wheats for drought adaptation. A genotype-dependent response to drought stress was observed for various drought-adaptive traits such as stomatal and osmotic regulation and antioxidant production, along with other morphological and physiological traits. Furthermore, genotypes with outstanding performance in terms of relative grain yield and associated physiological and morphological traits were identified and selected. The identified genotypes belonged to different taxa, regardless of their genetic groups. Overall, four genotypes, each from different species, including both wild types and landraces were selected as suitable drought-tolerant candidates. These candidates can be utilized in wheat improvement programs by identifying genes/alleles associated with specific drought tolerance characteristics in the identified drought-tolerant genotypes and incorporating those genes and traits into breeding programs.

Genome sequencing and comparative genomics help to identify genes/alleles linked to desirable traits (Bohra et al., 2022; Razzaq et al., 2021). However, integrating genes from CWRs into cultivated varieties presents challenges such as linkage drag, crossability barriers, infertility, low yield, and trait evaluation difficulties (Bohra et al., 2022; Farooq et al., 2025). Advances in modern breeding and genetic engineering provide solutions to these issues (Bohra et al., 2022; Shelake et al., 2022). Modern breeding, using techniques like CRISPR/Cas, enables *de novo* domestication by modifying key genes in wild species (Palmgren & Shabala, 2024; Yu & Li, 2022; Zeibig et al., 2024a). Genome assembly of wild wheats such as *Ae. tauschii*, *T. dicoccoides*, and *T. urartu* aids in *de novo* domestication in wheat (Bohra et al., 2022), along with information about domesticated genes related to traits like free-threshing, plant height, and grain number (Zeibig et al., 2024a). The identified drought-tolerant candidates, such as the genotype from the wild species *T. araraticum*, can be considered for *de novo* domestication. This species has also been found to be a suitable candidate for improving grain quality traits in wheat through *de novo* domestication (Zeibig et al., 2024b). Although *de novo* domestication of wild wheat is challenging, it expands access to genetic resources by utilizing them. *De novo* domesticated crops can serve as pre-breeding genetic sources. They can also be used in cultivation alongside modern crops for diversifying crop rotation and mixed cropping, thereby enriching agricultural biodiversity (Farooq et al., 2025). Furthermore, they can be cultivated on marginal lands with poor soil and minimal input, unlike modern cultivars, which usually require high-input conditions. This makes CWRs, landraces, and *de novo* domesticated crops ideal for sustainable farming systems. By enhancing biodiversity, they contribute to climate-resilient crop production.

However, incorporating *de novo* domesticated crops into crop cultivars may present compatibility challenges. Therefore, it is crucial that newly domesticated crops retain beneficial traits while adapting to farming systems.

6 Summary

Crop domestication and subsequent breeding have significantly enhanced global food security. However, these processes have also created genetic bottlenecks, reducing crops' genetic diversity and making them more vulnerable to environmental stresses. Crop wild relatives offer a diverse range of valuable traits, including resistance to both biotic and abiotic stresses, which can play a crucial role in enhancing food security amidst future climate change. Wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) is a crucial global staple crop, vital for food and energy supply, and thus plays a key role in food security. The *Triticum* gene pool of wheat includes diploid, tetraploid, and hexaploid forms. Wild wheats, which are exclusively diploid or tetraploid, serve as valuable genetic resources for wheat improvement due to their evolutionary relationships with domesticated wheat. Drought-induced wheat yield losses threaten global food security and are likely to increase with climate change. Drought stress impacts plant growth and development through morphological, physiological, and biochemical changes, ultimately reducing crop yield. To address the need for resilient crops in drought-prone regions, it is crucial to identify and develop varieties that exhibit drought tolerance traits. Although, wild wheat relatives harbor vast genetic diversity for drought tolerance traits, they have not been extensively explored. Therefore, the aim of this thesis was to explore and evaluate the drought-adaptive traits of wild *Triticum* taxa for use in breeding drought-tolerant wheat cultivars.

To evaluate drought tolerance traits in wild wheats and landraces, 110 genotypes from diverse *Triticum* taxa (60 wild wheats, 44 landraces and 6 modern cultivars) were evaluated at a drought-prone location with sandy loam soil at the research station of University of Giessen in Gross-Gerau during the 2021/22 winter wheat growing season. Twenty selected genotypes were further evaluated for physiological and morphological drought-adaptive traits in the same field during the 2022/23 season, and results were assessed across both years. Genotypes were selected based on their relative grain yield performance and associated physiological traits. Subsequently, a greenhouse experiment was conducted on 17 of these selected genotypes (11 wild wheats, 3 landraces, and 3 modern cultivars). This was followed by another greenhouse study focusing on 3 potential drought-tolerant genotypes from a neglected species, *T. araraticum*, and 2 modern bread wheat cultivars to evaluate in-depth physiological and biochemical traits that differ from modern bread wheat cultivars. These experiments were conducted under two different moisture conditions: irrigated and rainfed conditions in the field, and well-watered and drought conditions in the greenhouse experiments. In both field and greenhouse experiments, a genotype-dependent response to drought stress was observed. The *Triticum* gene pool showed wide variations in morphological, physiological and biochemical traits. Wild wheats such as *T. dicoccoides*,

T. urartu and *T. boeoticum* exhibited isohydric stomatal regulations, while landraces and modern cultivars demonstrated anisohydric stomatal regulations. Drought stress significantly increased the accumulation of osmoprotectants, such as proline and total soluble sugars, and enhanced antioxidant activity. Additionally, *T. araraticum* exhibited distinct drought tolerance via glutathione (GSH) redox balance, marked by enhanced enzymatic activities like monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR). In contrast, modern bread wheat cultivars showed enhanced ascorbate (AsA) redox in response to drought stress. Four drought-tolerant candidates have been selected from this study across four different taxa: three from wild and one from a landrace. These candidates can be used as pre-breeding sources for wheat improvement. Modern breeding techniques could be employed to incorporate drought-tolerant candidates into breeding program.

7 Zusammenfassung

Die Domestizierung von Nutzpflanzen und ihre anschließende Züchtung haben die weltweite Ernährungssicherheit erheblich verbessert. Diese Entwicklungen führten jedoch auch zu einem Verlust an genetischer Vielfalt, was die Kulturpflanzen anfälliger für Umwelteinflüsse macht. Wilde Verwandte von Nutzpflanzen stellen eine wichtige genetische Ressource dar, da sie viele nützliche Eigenschaften besitzen, darunter Resistenzen gegen biotische und abiotische Stressfaktoren, die entscheidend zur Ernährungssicherheit in Zeiten des Klimawandels beitragen können. Weizen (*Triticum aestivum* L. und *Triticum durum* Desf.) ist eine der wichtigsten Grundnahrungspflanzen weltweit und spielt eine zentrale Rolle für die Versorgung mit Lebensmitteln und Energie. Der *Triticum*-Genpool umfasst diploide, tetraploide und hexaploide Formen. Wilde Weizenarten sind ausschließlich diploid oder tetraploid und bilden aufgrund ihrer engen Verwandtschaft zum angebauten Weizen eine wertvolle Grundlage für die Züchtung. Ertragseinbußen durch Trockenheit gefährden die globale Ernährungssicherheit und werden durch den Klimawandel voraussichtlich noch zunehmen. Trockenstress beeinträchtigt das Pflanzenwachstum und die Entwicklung durch Veränderungen in der Morphologie, Physiologie und Biochemie, was letztlich zu geringeren Erträgen führt. Um den Bedarf an robusten Sorten in trockenen Regionen zu decken, ist die Züchtung von trockenheitstoleranten Weizensorten entscheidend. Wilde Weizenverwandte bieten hierfür eine große genetische Vielfalt, wurden aber bislang nur unzureichend erforscht. Ziel dieser Arbeit war es daher, die an Trockenheit angepassten Eigenschaften wilder *Triticum*-Taxa zu untersuchen, zu charakterisieren und zu bewerten, um ihr Potenzial für die Entwicklung trockenheitstoleranter Weizensorten zu nutzen.

Zur Untersuchung der Trockentoleranzmerkmale wurden 110 Genotypen verschiedener *Triticum*-Taxa (60 Wildformen, 44 Landrassen und 6 moderne Sorten) in der Vegetationsperiode 2021/22 an einem von Trockenheit betroffenen Standort mit sandigem Lehmboden auf dem Versuchsfeld der Universität Gießen in Groß-Gerau angebaut. Zwanzig ausgewählte Genotypen wurden in der Saison 2022/23 erneut auf demselben Feld auf ihre physiologischen und morphologischen Anpassungen an Trockenheit hin untersucht, und die Ergebnisse aus beiden Jahren wurden zusammen ausgewertet. Die Auswahl der Genotypen erfolgte auf Basis ihrer relativen Kornerträge und relevanter physiologischer Eigenschaften. Anschließend wurde ein Gewächshausversuch mit 17 dieser ausgewählten Genotypen durchgeführt (11 wilde Weizenarten, 3 Landsorten und 3 moderne Kultursorten). Darauf folgte eine weitere Gewächshausstudie, die sich auf drei potenziell trockenheitstolerante Genotypen einer vernachlässigten Art, *T. araraticum*, und zwei moderne Brotweizensorten konzentrierte, um deren physiologische und biochemische

Eigenschaften, die sich von denen moderner Brotweizensorten unterscheiden, eingehend zu untersuchen. Die Experimente wurden unter zwei verschiedenen Bewässerungsbedingungen durchgeführt: Bewässerung und Regenwasser im Feld sowie Bewässerung und Trockenheit im Gewächshaus. Anschließend wurde ein Gewächshausversuch mit 17 dieser Genotypen durchgeführt (11 Wildformen, 3 Landrassen und 3 moderne Sorten). Darauf folgte eine weitere Gewächshausstudie mit drei potenziell trockenoleranten Genotypen der wenig erforschten Art *T. araraticum* sowie zwei modernen Brotweizensorten, um deren physiologische und biochemische Merkmale im Detail zu untersuchen. Die Versuche wurden jeweils unter Bedingungen mit und ohne Trockenstress durchgeführt, auf dem Feld durch künstliche Beregnung sowie im Gewächshaus unter kontrollierten Bedingungen. In beiden Versuchen reagierten die Genotypen unterschiedlich auf Trockenstress. Der *Triticum*-Genpool zeigte große Unterschiede in morphologischen, physiologischen und biochemischen Merkmalen. Wilde Arten wie *T. dicocoides*, *T. urartu* und *T. boeoticum* besaßen isohydrische Stomata, während Landrassen und moderne Sorten überwiegend anisohydrische Stomata aufwiesen. Trockenstress erhöhte signifikant die Akkumulation von Osmoprotektoren wie Prolin und löslichen Zuckern und steigerte die antioxidativen Aktivitäten. Besonders *T. araraticum* zeigte eine ausgeprägte Trockentoleranz, die über das Glutathion (GSH) Redoxsystem vermittelt wurde und mit höheren Aktivitäten von Enzymen wie Monodehydroascorbat Reduktase (MDHAR) und Glutathion Reduktase (GR) verbunden war. Im Vergleich dazu zeigten moderne Brotweizensorten unter Trockenstress eine verstärkte Reaktion über das Ascorbat (AsA) Redoxsystem. In dieser Studie wurden vier trockenolerante Genotypen aus vier verschiedenen Taxa identifiziert – drei aus Wildformen und einer aus einer Landrasse, die als Ausgangsmaterial für die Weizenzüchtung geeignet sind. Moderne Züchtungsmethoden könnten eingesetzt werden, um diese Genotypen gezielt in Zuchtprogramme zu integrieren.

8 References

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Appendices

Appendix I: Supplementary materials from Pantha et al. (2024),

Chapter 2

Pantha, S., Kilian, B., Ozkan, H., Zeibig, F., Frei, M., 2025. A comparison of drought responses in wild wheat relatives and domesticated wheat grown under irrigated and rainfed field conditions. *Field Crops Research*, 321. <https://doi.org/10.1016/j.fcr.2024.109678>

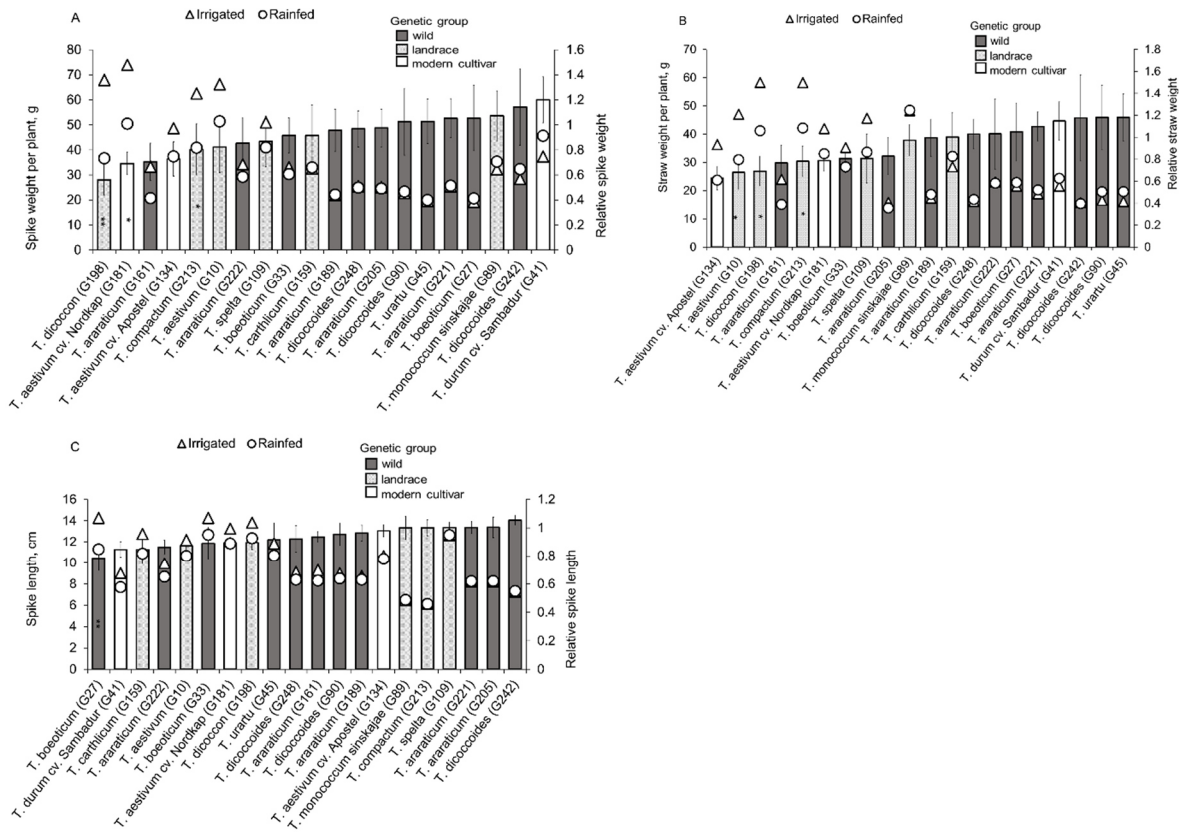


Figure S1: Effect of drought stress on yield-related traits in 20 wheat genotypes; spike weight per plant (A), straw weight per plant (B) and spike length (C). The primary vertical axis represents the means under rainfed and irrigated conditions. Open circles and triangles indicate means under rainfed and irrigated conditions respectively. The data are presented as average values over two growing seasons in 2022 and 2023. Mean relative values \pm standard errors of the means are shown on the secondary vertical axis represented by bars. The genotypes are arranged in ascending order from low relative values (left) to high relative value (right). The different shades indicate the genotypes' genetic groups. The asterisks inside the vertical bar indicate significant differences arising from the treatment effect (rainfed and irrigated conditions) for the same genotype (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

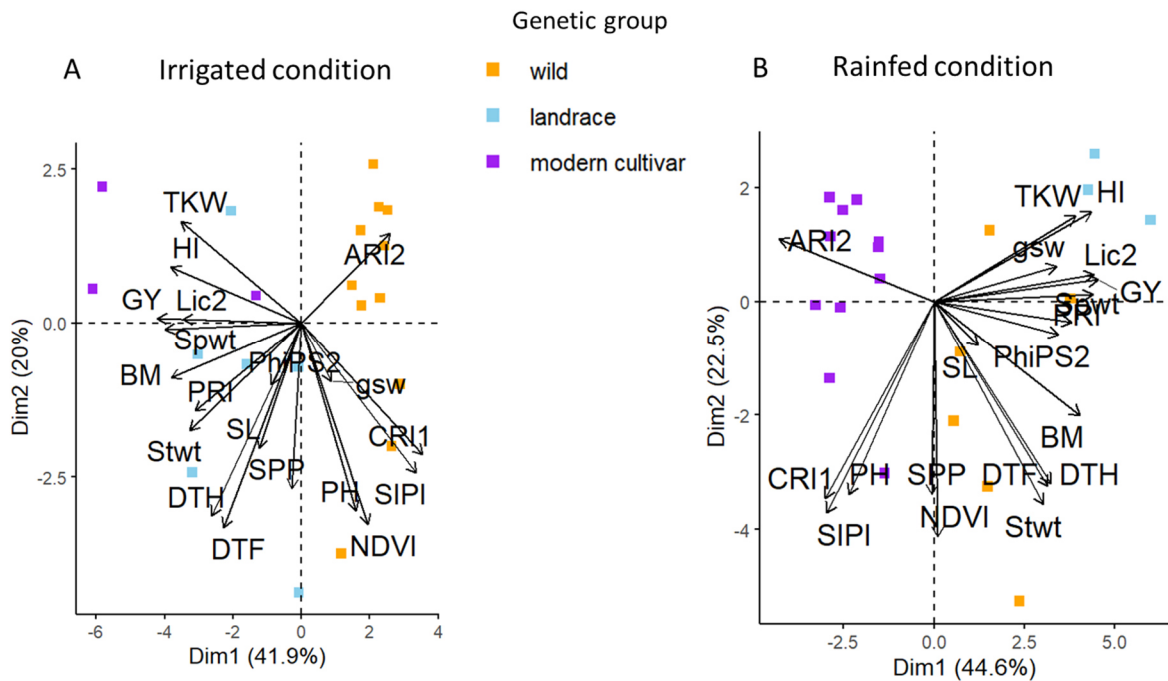


Figure S2: PCA biplot showing the distributions of the measured traits and individual genotypes under irrigated conditions (A) and rainfed conditions (B). DTH = days to heading, DTF = days to flowering, PH = plant height, SL = spike length, SPP = number of spikes per plant, Spwt = spike weight per plant, Stwt = straw weight per plant, BM = above ground biomass per plant, GY = grain yield per plant, TKW = thousand kernel weight, HI = harvest index, gsw = stomatal conductance, PhiPS2 = photosystem II efficiency, NDVI = normalized difference vegetation index, Lic2 = Lichtenthaler index 2, SIPI = structure insensitive pigment index, CRI1 = carotenoid reflectance index 1, PRI = photochemical reflectance index, and ARI2 = anthocyanin reflectance indices. Individual genotypes of wild status are shown in orange, genotypes of landrace status are shown in blue, and modern cultivars are shown in purple. Dim1 and Dim2 denote dimensions of PCA plot PC1 and PC2, respectively.

Table S1: List of 110 genotypes of wild wheats, landraces, and modern cultivars in the *Triticum* gene pool grown during the 2021/2022 season categorized by genetic group and taxa.

Genotype_id	Taxon	Genetic group	Seed source	Genome	Ploidy level	Original sample identifier	Other identifiers	Country of origin
G2	<i>T. dicoccoides</i>	wild	IPK	BA	4x	PI 654339		Turkey
G3	<i>T. aestivum</i>	landrace	IPK	BAD	6x	IG 45071		Oman
G5	<i>T. urartu</i>	wild	IPK	A ^u	2x	G 3246, PI 428320	ID 388 EP047-1	Lebanon
G7	<i>T. timopheevii</i>	landrace	IPK	GA ^t	4x	KU-1820	TR 106 TP041-TP045	former Soviet Union
G8	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 427365	PI 427365; TR 133-1; TA 101	Iran
G10	<i>T. aestivum</i>	landrace	IPK	BAD	6x	PI 367199		Afganistan
G12	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-1960		Turkey
G14	<i>T. araraticum</i>	wild	IPK	GAt	4x	TR 61 TP071	KU-8682	Iraq
G22	<i>T. dicoccoides</i>	wild	IPK	BA	4x	Gamla 1		Israel
G27	<i>T. boeoticum</i>	wild	IPK	A ^b	2x	PI 427583	ID 716 EP064-EP066	Turkey
G30	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-1982	2797	Turkey
G31	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8774	KU-8774; TR80-4; 8774	Iraq
G3	<i>T. boeoticum</i>	wild	IPK	A ^b	2x	PI 427458	ID 597 EP062-EP063	Turkey
G34	<i>T. carthlicum</i>	landrace	IPK	BA	4x	PI 573180		Turkey
G35	<i>T. vavilovii</i>	landrace	IPK	BAD	6x	TRI 9416		former Soviet Union
G36	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	HTRI 11356/76	TR 122; 2765	Azerbaijan
G38	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	IG 117891		Syria
G40	<i>T. boeoticum</i>	wild	IPK	A ^b	2x	PI 427629	ID 760 EP087-EP095	Turkey
G41	<i>T. durum</i> cv. Sambadur	modern cultivar	JLU	BA	4x	Hauptsaaften		
G44	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-8939	TR 99 TP083-TP087	Turkey
G45	<i>T. urartu</i>	wild	IPK	A ^u	2x	IG 45285		Syria
G47	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	2006-6-20-2-2 D	2633	Turkey
G52	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 297030	2566	Iraq
G57	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 221421	2554	
G59	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 290518	2565	Iraq
G60	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	IG 116165		Turkey
G61	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8682	TR 61 TP071; 2713	Iraq
G62	<i>T. boeoticum</i>	wild	IPK	A ^b	2x	PI 427963	ID 1089 EP003-EP004	Turkey
G65	<i>T. dicoccon</i>	landrace	IPK	BA	4x	TRI 17023	DEU146:W 7700	Turkey
G71	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 428018	3307; TR15	Turkey
G72	<i>T. karamyshevii</i>	landrace	IPK	BA	4x	01C0101162		former Soviet Union
G74	<i>T. turgidum</i>	landrace	IPK	BA	4x	PI 149812		China
G76	<i>T. sphaerococcum</i>	landrace	IPK	BAD	6x	PI 83402		China
G77	<i>T. dicoccon</i>	landrace	IPK	BA	4x	TRI 16880	DEU146:W 7561 B	Israel
G79	<i>T. urartu</i>	wild	IPK	A ^u	2x	G 3221, PI 428317	ID 393 EP048-1	Iran
G80	<i>T. spelta</i>	landrace	IPK	BAD	6x	TR 177		Spain
G83	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8691	2862	Iraq
G89	<i>T. monococcum</i> sinskajae	landrace	IPK	A ^m	2x	ID 69 SSD 2007+2009	Turkey	
G90	<i>T. dicoccoides</i>	wild	IPK	BA	4x	KU-8935		Turkey
G91	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8824A	1970-6-16-5-3-B-2	
G92	<i>T. durum</i>	landrace	IPK	BA	4x	DIC 169		France
G96	<i>T. karamyshevii</i>	landrace	IPK	BA	4x	KU-190-2		former Soviet Union
G99	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8945	TR 101-1; 8945	Iran
G100	<i>T. ispahanicum</i>	landrace	IPK	BA	4x	PI 352492		Iran

G103	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-8910	TR 94 TP077-TP079	Turkey
G105	<i>T. monococcum</i>	landrace	IPK	Am	2x	PI 277137	ID 1690	Greece
G107	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8912	2879	Iraq
G108	<i>T. zhukovskiyi</i>	landrace	IPK	GA ^t A ^b	6x	A TRI 12094/80	FAR 78; TR 156	Georgia
G109	<i>T. spelta</i>	landrace	IPK	BAD	6x	CGN08299		Iran
G111	<i>T. turgidum</i>	landrace	IPK	BA	6x	PI 502933		China
G112	<i>T. dicoccon</i>	landrace	IPK	BA	6x	PI 276002		Spain
G115	<i>T. aethiopicum</i>	landrace	IPK	BA	4x	KU-9388		Ethiopia
G116	<i>T. millitinae</i>	landrace	IPK	GA ^t	4x	01C0202037		former Soviet Union
G118	<i>T. dicoccoides</i>	wild	IPK	BA	4x	PI 352322	DIC 44 TP011-TP020	Lebanon
G121	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	PI 538461	TR 17 TP061-TP063	Iraq
G122	<i>T. dicoccon</i>	landrace	IPK	BA	4x	TRI 5860		Iran
G126	<i>T. carthlicum</i>	landrace	IPK	BA	4x	TRI 15127		Georgia
G127	<i>T. spelta</i>	landrace	IPK	BAD	6x	TRI 9681		Morocco
G128	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8682	TR 61; 8682	Iraq
G131	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 427357	TR4	Iraq
G134	<i>T. aestivum</i> cv. Apostel	modern cultivar	JLU	BAD	6x	IG Pflanzenzucht		
G135	<i>T. polonicum</i>	landrace	IPK	BA	4x	TRI 3478		Ethiopia
G137	<i>T. spelta</i>	landrace	IPK	BAD	6x	PI 355626		Germany
G139	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-8713	TR 69 TP072-TP076	Iraq
G140	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-8945	TR 101-1	Iran
G142	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8925	1970-6-28-3-5-A-2; 2892	Iraq
G143	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8640	TR 56-1; 8640	Iraq
G146	<i>T. aestivum</i> cv. Julius	modern cultivar	JLU	BAD	6x	KWS SAAT SE &Co.		
G154	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	TRI 16599	2399/3	Turkey
G156	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8939	2753; TR99	Turkey
G159	<i>T. carthlicum</i>	landrace	IPK	BA	4x	TRI 3426		Iran
G160	<i>T. aestivum</i>	landrace	IPK	BAD	6x	CGN12270		Iran
G161	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8709	TR 67; 8709	Iraq
G162	<i>T. petropavlovskiyi</i>	landrace	IPK	BAD	6x	MVGB000567		Unknown
G164	<i>T. polonicum</i>	landrace	IPK	BA	4x	ID 597 EP062-EP063		Turkey
G166	<i>T. urartu</i>	wild	IPK	A ^u	2x	PI 654310		Turkey
G167	<i>T. durum</i> cv. Wintergold	modern cultivar	JLU	BA	4x	SAATEN UNION		
G169	<i>T. aestivum</i> cv. RGT Reform	modern cultivar	JLU	BAD	6x	RAGT Saaten		
G171	<i>T. spelta</i>	landrace	IPK	BAD	6x	CGN08304		Iran
G174	<i>T. monococcum</i>	landrace	IPK	A ^m	2x	LPCH 95	ID 1737	Morocco
G175	<i>T. macha</i>	landrace	IPK	BAD	6x	PI 140191		Iran
G179	<i>T. turanicum</i>	landrace	IPK	BA	4x	TRI 10343		Uzbekistan
G181	<i>T. aestivum</i> cv. Nordkap	modern cultivar	JLU	BAD	6x	SAATEN UNION		
G185	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	2005-7-5-9-4	3129	Turkey
G186	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 427371		Iran
G189	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	HTRI 11509/83	TR 133-1; 2776	Iran
G190	<i>T. vavilovii</i>	landrace	IPK	BAD	6x	TRI 11554		former Soviet Union
G192	<i>T. monococcum</i>	landrace	IPK	A ^m	2x	PI 191097	ID 1681	Spain
G197	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	8872	TR89	Iraq
G198	<i>T. dicoccon</i>	landrace	IPK	BA	4x	PI 352348		Syria
G200	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	KU-1902		Armenia
G203	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-1980A	1976-8-14-1-39; 2913	Iraq
G205	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8733	8733	Iraq
G208	<i>T. dicoccon</i>	landrace	IPK	BA	4x	PI 470739		Turkey
G210	<i>T. durum</i>	landrace	IPK	BA	4x	DIC 193		Italy
G213	<i>T. compactum</i>	landrace	IPK	BAD	6x	PI 60740		Egypt
G215	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-1933	1976-7-27-2-yo; 2788	Iraq
G216	<i>T. sphaerococcum</i>	landrace	IPK	BAD	6x	PI 42014		India
G217	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	2006-6-20-11-6 D		Turkey
G218	<i>T. dicoccon</i>	landrace	IPK	BA	4x	TRI 10324		Slovakia
G219	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8939	TR 99; 8939	Turkey
G221	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8866	1970-6-17-1-4-8-2; 2873	Iraq

G222	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8545	TR 51;8545	Iraq
G232	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8938	TR 98-1-1; 2749	Turkey
G233	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	PI 596290		Turkey
G234	<i>T. turgidum</i>	landrace	IPK	BA	4x	PI 572918		China
G238	<i>T. araraticum</i>	wild	Çukurova University	GA ^t	4x	KU-8529	8529	Iraq
G242	<i>T. dicoccoides</i>	wild	IPK	BA	4x	KU-14474		Israel
G244	<i>T. araraticum</i>	wild	IPK	GA ^t	4x	IG 119456		Syria
G248	<i>T. dicoccoides</i>	wild	IPK	BA	4x	KU-8812		Iraq

Table S2: Mean grain yield per plant and aboveground biomass per plant and ranking of genotypes by relative values of grain yield and above ground biomass in 110 genotypes of wild wheats, landraces, and modern cultivars in the *Triticum* gene pool grown during the 2021/2022 season.

Genotype _id	Taxon	Genetic group	Mean grain yield per plant, g		Rank by relative grain yield	Mean aboveground biomass per plant, g		Rank by relative aboveground biomass
			Irrigated	Rainfed		Irrigated	Rainfed	
G2	<i>T. dicoccoides</i>	wild	9.5	8.0	28	56.5	32.2	76
G3	<i>T. aestivum</i>	landrace	45.1	17.7	101	120.7	59.3	96
G5	<i>T. urartu</i>	wild	12.6	7.2	69	55.6	30.8	81
G7	<i>T. timopheevii</i>	landrace	35.8	15.5	97	119.7	60.3	93
G8	<i>T. araraticum</i>	wild	7.8	2.8	104	36.8	12.4	109
G10	<i>T. aestivum</i>	landrace	50.6	25.0	83	127.2	60.3	98
G12	<i>T. araraticum</i>	wild	15.2	8.9	64	61.8	36.7	70
G14	<i>T. araraticum</i>	wild	12.2	9.0	41	49.4	41.4	27
G22	<i>T. dicoccoides</i>	wild	11.8	6.6	72	58.4	43.0	41
G27	<i>T. boeoticum</i>	wild	6.8	5.6	32	37.1	33.7	22
G30	<i>T. araraticum</i>	wild	18.9	11.9	57	69.6	47.7	53
G31	<i>T. araraticum</i>	wild	4.0	2.2	76	26.4	16.2	65
G33	<i>T. boeoticum</i>	wild	12.9	12.0	20	71.2	55.7	35
G34	<i>T. carthlicum</i>	landrace	12.6	5.9	88	33.9	18.4	83
G35	<i>T. vavilovii</i>	landrace	44.7	37.2	31	148.3	124.5	26
G36	<i>T. araraticum</i>	wild	5.8	2.9	81	27.5	17.6	60
G38	<i>T. araraticum</i>	wild	10.7	9.3	26	56.5	38.7	54
G40	<i>T. boeoticum</i>	wild	17.8	10.6	61	66.7	34.3	92
G41	<i>T. durum</i> cv. Sambadur	modern cultivar	30.4	46.5	3	62.3	86.4	3
G44	<i>T. araraticum</i>	wild	15.4	10.1	51	64.5	49.1	39
G45	<i>T. urartu</i>	wild	10.7	8.6	35	34.2	28.2	30
G47	<i>T. araraticum</i>	wild	15.1	8.2	74	57.9	30.4	87
G52	<i>T. araraticum</i>	wild	10.7	3.7	105	41.2	17.9	101
G57	<i>T. araraticum</i>	wild	11.2	7.7	43	48.7	30.9	63
G59	<i>T. araraticum</i>	wild	9.1	5.6	58	43.9	22.8	90
G60	<i>T. araraticum</i>	wild	12.8	5.4	98	56.8	30.3	86
G61	<i>T. araraticum</i>	wild	11.0	4.9	93	42.4	18.6	100
G62	<i>T. boeoticum</i>	wild	8.9	3.3	102	67.1	16.6	110
G65	<i>T. dicoccon</i>	landrace	28.6	22.1	36	66.2	53.0	33
G71	<i>T. araraticum</i>	wild	6.9	3.1	94	43.0	25.5	71
G72	<i>T. karamyshevii</i>	landrace	33.5	10.7	106	100.0	54.4	82
G74	<i>T. turgidum</i>	landrace	28.5	20.6	42	69.5	53.9	37
G76	<i>T. sphaerococcum</i>	landrace	31.1	17.6	70	66.9	39.5	72
G77	<i>T. dicoccon</i>	landrace	47.5	35.0	40	146.8	106.8	44

G79	<i>T. urartu</i>	wild	9.5	4.9	79	36.3	22.0	67
G80	<i>T. spelta</i>	landrace	75.9	88.9	9	200.8	223.3	8
G83	<i>T. araraticum</i>	wild	7.7	2.4	108	30.4	12.3	106
G89	<i>T. monococcum</i> sinskajae	landrace	19.8	24.1	8	92.4	96.5	6
G90	<i>T. dicoccoides</i>	wild	8.8	7.3	30	36.7	34.9	16
G91	<i>T. araraticum</i>	wild	11.1	3.5	107	52.7	22.8	102
G92	<i>T. durum</i>	landrace	30.0	24.2	34	65.0	55.6	25
G96	<i>T. karamyshevii</i>	landrace	48.8	31.3	55	146.1	82.0	78
G99	<i>T. araraticum</i>	wild	8.6	4.1	85	35.1	18.3	89
G100	<i>T. ispahanicum</i>	landrace	25.8	17.3	46	69.9	44.5	61
G103	<i>T. araraticum</i>	wild	16.3	7.5	89	68.5	36.6	85
G105	<i>T. monococcum</i>	landrace	27.0	26.3	15	90.8	81.0	23
G107	<i>T. araraticum</i>	wild	7.3	4.7	52	32.7	21.0	59
G108	<i>T. zhukovskyi</i>	landrace	9.9	5.8	66	55.1	38.2	51
G109	<i>T. spelta</i>	landrace	36.8	16.1	96	111.0	43.4	107
G111	<i>T. turgidum</i>	landrace	35.9	22.6	56	96.6	70.7	42
G112	<i>T. dicoccon</i>	landrace	36.7	38.9	47	90.1	95.7	11
G115	<i>T. aethiopicum</i>	landrace	11.6	6.7	65	44.8	26.1	74
G116	<i>T. militinae</i>	landrace	15.0	6.2	99	67.7	28.1	103
G118	<i>T. dicoccoides</i>	wild	24.3	10.8	95	93.0	59.0	62
G121	<i>T. araraticum</i>	wild	3.7	3.3	21	15.6	12.6	31
G122	<i>T. dicoccon</i>	landrace	31.4	29.5	19	87.5	69.1	34
G126	<i>T. carthlicum</i>	landrace	12.7	15.9	7	36.5	40.2	9
G127	<i>T. spelta</i>	landrace	72.2	60.5	29	178.2	148.8	29
G128	<i>T. araraticum</i>	wild	5.1	2.8	73	23.5	13.5	75
G131	<i>T. araraticum</i>	wild	5.1	2.4	87	19.8	11.0	80
G134	<i>T. aestivum</i> cv. Apostel	modern cultivar	32.9	13.1	100	73.6	30.2	104
G135	<i>T. polonicum</i>	landrace	32.5	18.6	68	79.0	46.3	73
G137	<i>T. spelta</i>	landrace	52.9	32.4	60	140.9	91.1	58
G139	<i>T. araraticum</i>	wild	8.6	5.5	54	36.0	24.7	52
G140	<i>T. araraticum</i>	wild	7.4	5.0	44	29.9	19.9	55
G142	<i>T. araraticum</i>	wild	5.3	3.6	45	21.4	14.9	49
G143	<i>T. araraticum</i>	wild	8.1	4.1	82	33.2	16.3	97
G146	<i>T. aestivum</i> cv. Julius	modern cultivar	40.0	50.3	6	92.0	108.3	5
G154	<i>T. araraticum</i>	wild	12.8	11.2	23	47.2	35.6	40
G156	<i>T. araraticum</i>	wild	14.8	14.5	14	63.5	66.8	12
G159	<i>T. carthlicum</i>	landrace	31.4	30.7	13	85.5	92.6	10
G160	<i>T. aestivum</i>	landrace	54.5	52.6	16	117.6	119.3	14
G161	<i>T. araraticum</i>	wild	7.7	7.3	17	30.8	29.3	17
G162	<i>T. petropavlovskyi</i>	landrace	60.3	45.6	37	205.2	135.2	56
G164	<i>T. polonicum</i>	landrace	41.4	36.2	24	105.5	95.8	21
G166	<i>T. urartu</i>	wild	13.6	15.7	10	55.2	62.1	7
G167	<i>T. durum</i> cv. Wintergold	modern cultivar	35.6	20.0	71	76.3	43.4	77
G169	<i>T. aestivum</i> cv. RGT Reform	modern cultivar	43.8	38.2	25	80.0	75.2	18
G171	<i>T. spelta</i>	landrace	46.7	41.3	22	148.6	104.1	48
G174	<i>T. monococcum</i>	landrace	13.6	14.2	12	48.5	50.4	13

G175	<i>T. macha</i>	landrace	31.6	14.1	92	109.4	51.8	99
G179	<i>T. turanicum</i>	landrace	31.6	23.3	39	77.0	59.4	38
G181	<i>T. aestivum</i> cv. Nordkap	modern cultivar	64.7	29.0	75	130.2	65.4	64
G185	<i>T. araraticum</i>	wild	4.0	8.5	2	39.8	32.0	32
G186	<i>T. araraticum</i>	wild	12.6	8.4	49	55.3	33.6	66
G189	<i>T. araraticum</i>	wild	7.1	5.8	33	31.7	26.5	28
G190	<i>T. vavilovii</i>	landrace	83.1	41.1	84	210.9	104.1	94
G192	<i>T. monococcum</i>	landrace	5.3	16.0	1	33.7	60.7	1
G197	<i>T. araraticum</i>	wild	13.6	8.9	50	57.2	40.8	47
G198	<i>T. dicoccon</i>	landrace	42.2	15.2	103	157.5	95.0	68
G200	<i>T. araraticum</i>	wild	8.1	5.4	48	30.2	19.9	57
G203	<i>T. araraticum</i>	wild	9.2	2.9	109	36.4	13.1	108
G205	<i>T. araraticum</i>	wild	6.4	6.9	11	29.4	25.3	24
G208	<i>T. dicoccon</i>	landrace	23.8	12.3	78	61.0	32.6	84
G210	<i>T. durum</i>	landrace	27.1	16.8	59	81.3	63.2	36
G213	<i>T. compactum</i>	landrace	55.4	25.3	91	146.2	81.9	79
G215	<i>T. araraticum</i>	wild	17.4	10.3	63	70.4	51.0	45
G216	<i>T. sphaerococcum</i>	landrace	13.8	8.9	53	37.7	26.9	46
G217	<i>T. araraticum</i>	wild	9.4	5.6	62	47.9	25.1	88
G218	<i>T. dicoccon</i>	landrace	21.8	20.5	18	55.0	50.1	20
G219	<i>T. araraticum</i>	wild	21.4	9.8	90	92.5	45.5	95
G221	<i>T. araraticum</i>	wild	6.0	7.7	5	32.8	39.4	4
G222	<i>T. araraticum</i>	wild	12.5	10.6	27	48.3	47.8	15
G232	<i>T. araraticum</i>	wild	17.9	3.0	110	63.0	32.7	91
G233	<i>T. araraticum</i>	wild	11.7	6.8	67	52.0	36.1	50
G234	<i>T. turgidum</i>	landrace	57.1	27.2	86	131.6	96.0	43
G238	<i>T. araraticum</i>	wild	14.7	7.5	80	74.2	30.3	105
G242	<i>T. dicoccoides</i>	wild	8.7	12.9	4	38.3	56.2	2
G244	<i>T. araraticum</i>	wild	18.4	9.8	77	88.5	52.9	69
G248	<i>T. dicoccoides</i>	wild	8.9	6.7	38	32.7	30.6	19

Table S3: The mean values of yield-related traits measured for 20 genotypes and averaged across two growing seasons, 2021/2022 and 2022/2023, (Gross-Gerau, Germany).

Genotype_id	Taxon	Genetic group	GY/plant: irrigated (g)	GY/plant: rainfed (g)	BM/plant: irrigated (g)	BM/plant: rainfed (g)	HI: irrigated	HI: rainfed	TKW: irrigated (g)	TKW: rainfed (g)
G10	<i>T. aestivum</i>	landrace	46.2	33.0	113.3	82.5	0.40	0.39	40.2	37.5
G27	<i>T. boeoticum</i>	wild	8.5	9.1	40.8	43.5	0.20	0.21	13.5	13.2
G33	<i>T. boeoticum</i>	wild	13.7	12.1	68.4	58.7	0.20	0.19	13.4	12.0
G41	<i>T. durum</i> cv.	modern cultivar	27.2	36.1	58.9	70.0	0.45	0.50	41.7	45.0
G45	<i>T. urartu</i>	wild	10.5	11.2	35.8	39.6	0.26	0.27	11.6	11.6
G89	<i>T. monococcum sinskajae</i>	landrace	18.9	22.1	80.6	83.8	0.23	0.26	25.7	25.9
G90	<i>T. dicoccoides</i>	wild	9.6	9.5	39.4	43.0	0.23	0.20	26.2	25.6
G109	<i>T. spelta</i>	landrace	33.4	22.0	96.6	74.7	0.33	0.35	39.0	36.2
G134	<i>T. aestivum</i> cv.	modern cultivar	38.1	24.3	85.0	56.4	0.44	0.42	41.3	40.5
G159	<i>T. carthlicum</i>	landrace	22.3	22.3	60.9	65.1	0.37	0.33	30.8	27.8
G161	<i>T. araraticum</i>	wild	13.2	8.1	57.3	35.8	0.24	0.42	26.0	22.8
G181	<i>T. aestivum</i> cv.	modern cultivar	53.6	37.7	116.0	83.6	0.48	0.45	41.6	38.5
G189	<i>T. araraticum</i>	wild	8.7	8.7	39.3	40.8	0.22	0.21	24.3	23.7
G198	<i>T. dicoccon</i>	landrace	36.1	16.5	126.3	77.9	0.28	0.24	40.5	40.0
G205	<i>T. araraticum</i>	wild	11.0	10.2	40.9	38.4	0.21	0.25	28.5	27.6
G213	<i>T. compactum</i>	landrace	44.2	23.8	120.9	83.1	0.34	0.32	42.7	38.7
G221	<i>T. araraticum</i>	wild	10.1	9.9	44.2	46.0	0.22	0.20	23.2	22.2
G222	<i>T. araraticum</i>	wild	14.2	12.3	57.3	51.9	0.24	0.20	25.6	25.2
G242	<i>T. dicoccoides</i>	wild	8.7	10.8	44.1	47.8	0.19	0.22	29.7	32.3
G248	<i>T. dicoccoides</i>	wild	11.0	10.4	41.7	41.8	0.27	0.23	32.5	28.8

BM = aboveground biomass per plant, GY = grain yield per plant, TKW = thousand kernel weight, and HI = harvest index.

Table S3 (continued): The mean values of physiological and morphological traits for 20 genotypes and averaged across two growing seasons, 2021/2022 and 2022/2023 (Gross-Gerau, Germany).

Genotype _id	Taxon	Genetic group	Stomatal conductance: irrigated (mmol m ⁻² s ⁻¹)	Stomatal conductance: rainfed (mmol m ⁻² s ⁻¹)	Plant height: irrigated (cm)	Plant height: rainfed (cm)	Number of spikes /plant: irrigated	Number of spikes /plant: rainfed
G10	<i>T. aestivum</i>	landrace	145.1	57.3	94.2	93.2	36.5	24.0
G27	<i>T. boeoticum</i>	wild	126.2	59.6	136.8	121.7	18.4	19.1
G33	<i>T. boeoticum</i>	wild	135.5	47.7	134.6	124.8	27.9	29.8
G41	<i>T. durum</i> cv.	modern cultivar	115.8	78.8	79.6	72.2	11.3	13.6
G45	<i>T. urartu</i>	wild	153.2	40.6	105.8	106.1	23.0	27.9
G89	<i>T. monococcum</i> sinskajae	landrace	103.6	70.2	101.8	100.2	59.0	59.7
G90	<i>T. dicoccoides</i>	wild	95.7	51.9	94.2	89.8	17.5	19.1
G109	<i>T. spelta</i>	landrace	74.6	66.1	98.3	94.9	24.0	22.3
G134	<i>T. aestivum</i> cv.	modern cultivar	96.4	74.8	73.9	75.1	17.9	15.5
G159	<i>T. carthlicum</i>	landrace	106.1	52.5	106.9	93.6	19.1	20.9
G161	<i>T. araraticum</i>	wild	76.7	42.3	106.4	96.4	24.5	17.1
G181	<i>T. aestivum</i> cv.	modern cultivar	92.1	67.4	78.2	75.2	19.6	15.8
G189	<i>T. araraticum</i>	wild	96.5	69.2	98.9	98.4	21.1	18.9
G198	<i>T. dicoccon</i>	landrace	78.4	37.9	117.0	109.5	20.8	14.9
G205	<i>T. araraticum</i>	wild	73.4	52.3	94.2	96.9	16.1	17.9
G213	<i>T. compactum</i>	landrace	78.4	59.7	106.2	108.5	23.6	19.6
G221	<i>T. araraticum</i>	wild	86.1	35.2	89.6	98.4	21.8	24.6
G222	<i>T. araraticum</i>	wild	62.7	21.5	95.4	88.1	23.4	21.9
G242	<i>T. dicoccoides</i>	wild	137.4	57.4	89.9	81.5	16.1	16.9
G248	<i>T. dicoccoides</i>	wild	142.0	57.3	85.1	82.0	19.8	21.8

Table S4: The mean value of physiological and morphological traits by genetic groups averaged across two growing seasons: 2022 and 2023 (Gross-Gerau, Germany).

Genetic group and ploidy	Days to heading		Days to flowering		gsw, mmol m ⁻² s ⁻¹		PhiPS2		NDVI		Lic2		CRI1	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
Wild	217	216	225	224	107.5	50.0	0.41	0.40	0.57	0.51	0.81	0.74	2.77	2.42
Diploid	220	219	228	226	138.3	49.3	0.47	0.33	0.61	0.56	0.85	0.78	3.00	2.75
Tetraploid	216	215	224	224	98.9	50.2	0.40	0.42	0.56	0.50	0.80	0.72	2.67	2.28
Landrace	225	224	233	232	97.7	57.2	0.43	0.46	0.57	0.54	0.84	0.87	2.63	2.42
Diploid	233	233	246	244	103.6	70.2	0.41	0.56	0.61	0.60	0.84	0.85	3.37	2.98
Tetraploid	227	228	235	236	92.2	45.2	0.48	0.44	0.57	0.59	0.84	0.86	2.64	2.96
Hexaploid	221	220	228	226	99.4	61.0	0.39	0.44	0.55	0.50	0.85	0.89	2.38	1.94
Modern cultivar	224	224	232	232	101.4	73.7	0.45	0.47	0.55	0.49	1.07	1.14	1.57	1.04
Tetraploid	223	220	231	229	115.8	78.8	0.45	0.51	0.60	0.51	0.96	1.02	2.52	1.67
Hexaploid	225	225	233	233	94.3	71.1	0.45	0.45	0.53	0.48	1.12	1.21	1.09	0.72

gsw = stomatal conductance, PhiPS2 = photosystem II efficiency, NDVI = normalized difference vegetation index, Lic2 = Lichtenthaler index 2, CRI1 = carotenoid reflectance index 1.

Table S4 (continued): The mean value of physiological and morphological traits by genetic groups across two growing seasons, 2022 and 2023 (Gross-Gerau, Germany).

Genetic group and ploidy	Aboveground biomass per plant, g		Grain yield per plant, g		Harvest index		1000-kernel weight, g		Number of spikes per plant		Spike length, cm		Plant height, cm	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Irrigated	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
Wild	46.1	44.3	10.8	10.2	0.23	0.22	10.1	10.1	10.8	10.2	10.1	9.2	102.3	98.7
Diploid	47.4	47.3	10.8	10.8	0.22	0.22	13.6	13.6	10.8	10.8	13.6	11.6	124.8	117.5
Tetraploid	45.7	43.2	10.9	10.0	0.23	0.21	8.8	8.8	10.9	10.0	8.8	8.3	94.2	91.4
Landrace	99.8	78.0	33.5	23.3	0.32	0.31	10.7	10.7	33.5	23.3	10.7	9.9	104.0	100.1
Diploid	80.6	83.8	18.9	22.1	0.23	0.26	6.5	6.5	18.9	22.1	6.5	6.5	101.8	100.2
Tetraploid	93.6	71.9	29.2	19.2	0.33	0.28	13.3	13.3	29.2	19.2	13.3	11.7	111.9	102.1
Hexaploid	110.3	80.1	41.3	26.3	0.36	0.35	10.3	10.3	41.3	26.3	10.3	9.8	99.6	98.9
Modern cultivar	86.6	70.0	39.6	32.7	0.46	0.46	11.0	11.0	39.6	32.7	11.0	10.0	77.2	74.2
Tetraploid	58.9	70.0	27.2	36.1	0.45	0.50	9.0	9.0	27.2	36.1	9.0	7.7	79.6	72.2
Hexaploid	100.5	70.0	45.8	31.0	0.46	0.43	12.1	12.1	45.8	31.0	12.1	11.1	76.1	75.1

Appendix II: Supplementary materials from Pantha et al. (2024),

Chapter 3

Pantha, S., Kilian, B., Özkan, H., Zeibig, F., Frei, M., 2024. Physiological and biochemical changes induced by drought stress during the stem elongation and anthesis stages in the *Triticum* genus. *Environmental and Experimental Botany*, 228. <https://doi.org/10.1016/j.envexpbot.2024.106047>

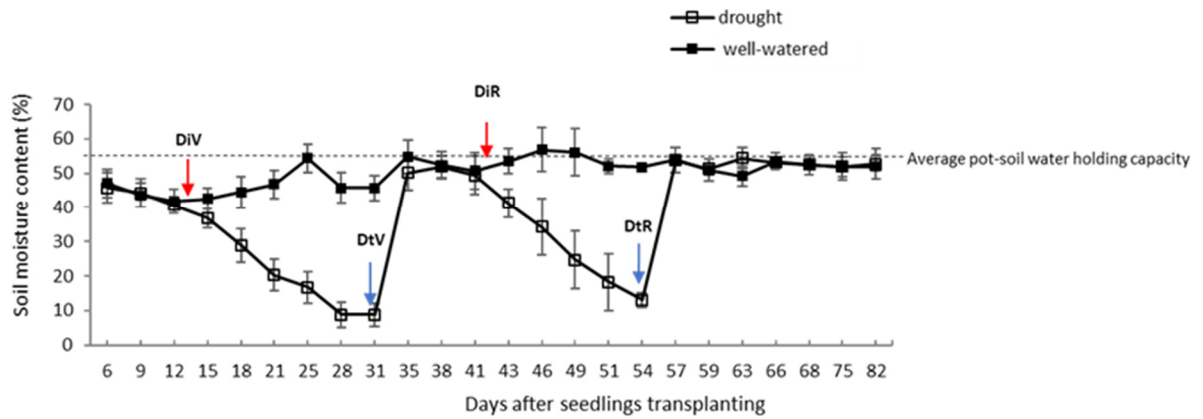


Figure S1: Average soil moisture content under well-watered and drought conditions measured on different days after transplanting of wheat seedlings in pots in the greenhouse conditions. Bars indicate average \pm standard deviations, red arrows indicate the time point of drought initiation, and blue arrows indicate the time point of drought termination. The straight dotted line indicates the average pot soil water-holding capacity. DiV = drought initiation at the stem elongation stage, DtV = drought termination at the stem elongation stage, DiR = drought initiation at the anthesis stage, and DtR = drought termination at the anthesis stage.

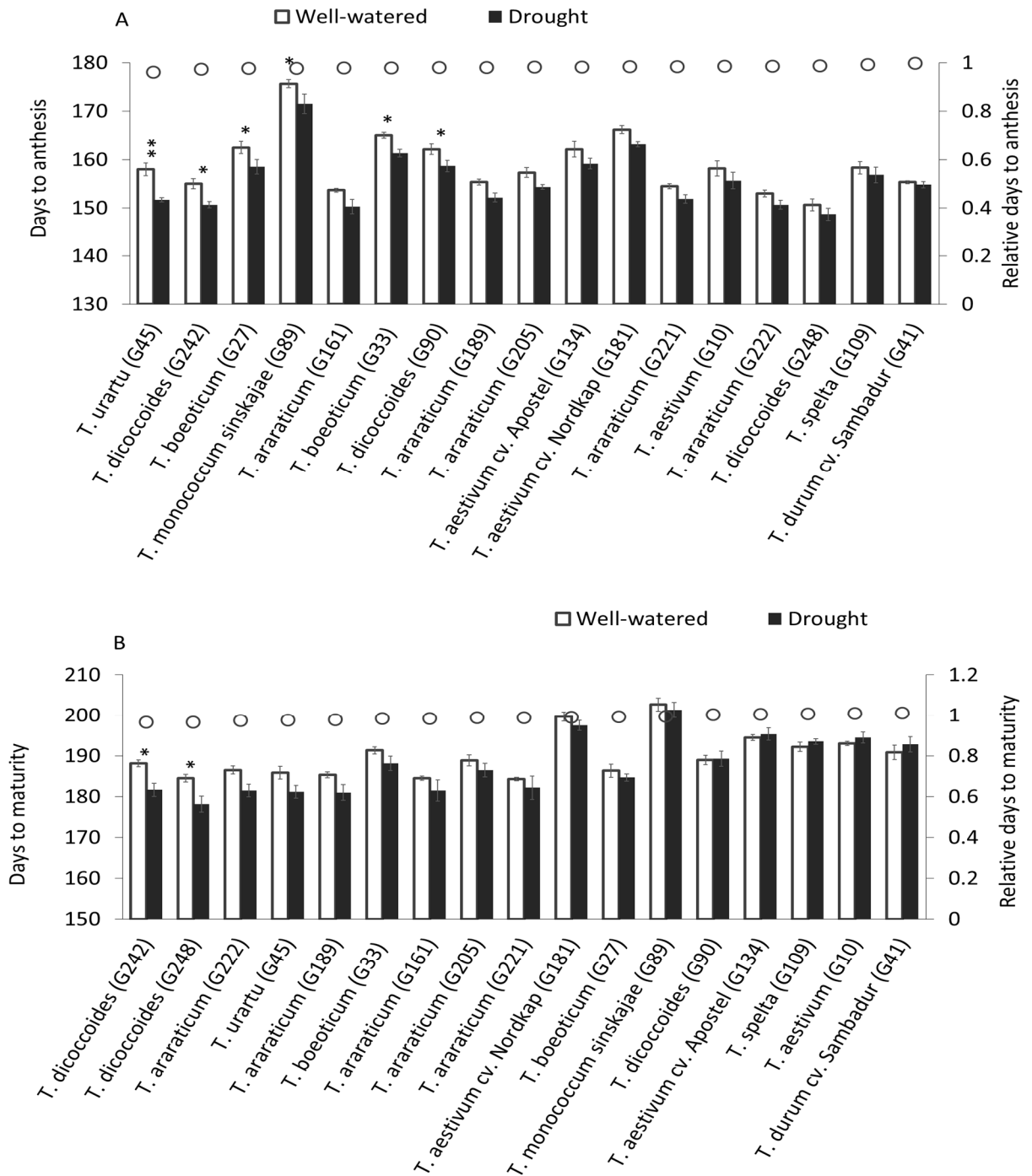


Figure S2: Days to anthesis (A) and days to maturity (B). Unshaded circles indicate mean relative values (drought/control) on the secondary vertical axis, with genotypes arranged in ascending order from low relative values (to the left) to high relative values (to the right). White and black vertical bars indicate mean values under well-watered and drought conditions, respectively, on the primary vertical axis. Data are average values of six replicates \pm standard errors. Asterisks above the vertical bars indicate significant differences between the well-watered and drought conditions (treatment effect) for the same genotype via pairwise comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Table S3: Status-wise mean values of the measured parameters for wild, landrace, and modern cultivars under two different treatment conditions at the stem elongation stage (greenhouse experiment 2022/23).

Traits	Status-wise mean					
	Wild		Landrace		Modern cultivar	
	Well-watered	Drought	Well-watered	Drought	Well-watered	Drought
Relative water content (%)	91.4 ^b	63.4 ^a	91.4 ^b	61.4 ^a	93.0 ^b	56.8 ^a
Malondialdehyde (nmol/ml/g, FW)	15.3 ^a	25.3 ^b	16.9	23.6	20	26.4
Proline ($\mu\text{g/g}$, FW)	56.9 ^a	3945.2 ^b	54.8 ^a	3563.5 ^b	54.4 ^a	4623.9 ^b
Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)	0.47 ^b	0.06 ^a	0.29 ^b	0.06 ^a	0.34 ^b	0.03 ^a
PhiPS2	0.62	0.65	0.69	0.67	0.64	0.64
NDVI	0.59	0.58	0.6	0.58	0.62	0.62
MCARI1	2.04	2.35	1.83	2.23	2.07	2.46
Lic2	0.97	0.99	0.96	1	0.97	1.02
ARI2	-0.21	-0.24	-0.18	-0.22	-0.23	-0.31
CRI2	1.59	1.27	1.58	1.15	1.42	1.17
Shoot length (cm)	69.6 ^b	56.9 ^a	71	58.9	67.1	55
Total tillers	13 ^b	9 ^a	13 ^b	8 ^a	12 ^b	7 ^a

FW = fresh weight; PhiPS2 = photosystem II efficiency; NDVI = normalized difference vegetation index; Lic2 = Lichtenthaler Index; CRI2 = carotenoid reflectance index 2; MCARI1 = modified chlorophyll absorption in reflectance index 1; ARI2 = anthocyanin reflectance index 2. The superscript letters following the mean values within the same status group for the same trait indicate the significance of the treatment effect within the same status group at $p < 0.05$ by emmeans pairwise comparison.

Table S4: Status-wise mean values of the measured parameters for wild, landrace, and modern cultivars under two different treatment conditions at the anthesis stage (greenhouse experiment 2022/23).

Traits	Mean status-wise					
	Wild		Landrace		Modern cultivars	
	Well-watered	Drought	Well-watered	Drought	Well-watered	Drought
Days to heading	151 ^b	148 ^a	158	156	155	151
Days to anthesis	157 ^b	154 ^a	164	161	161	159
Days to maturity	187 ^b	183 ^a	196	196	195	195
Plant height (cm)	130.2 ^b	95.0 ^a	116.4 ^b	91.7 ^a	81.3	72.2
Spike length (cm)	10	9	10.2	9.3	10.6	10.7
Peduncle exertion length (cm)	32.3 ^b	20.2 ^a	21.3 ^b	13.7 ^a	9.9	8
Effective tillers	17	14	23	20	12	8
Above ground biomass (g)	33.0 ^b	15.6 ^a	48.9 ^b	26.8 ^a	40.9 ^b	22.6 ^a
Grain yield (g)	8.5 ^b	4.3 ^a	19.0 ^b	8.7 ^a	21.0 ^b	10.0 ^a
Harvest index (%)	26.1	27.9	36.1	32.2	50.6 ^b	43.6 ^a
Grain number	443.8 ^b	238.0 ^a	443.4 ^b	271.1 ^a	477.4 ^b	226.8 ^a
Thousand kernel weight (g)	23.1	22.4	40.2	33.6	44	43.8
Relative water content (%)	87.7 ^b	62.9 ^a	91.1	70.4	92.4	67.1
Malondialdehyde (nmol/ml/g, FW)	10.1 ^a	15.9 ^b	11.3 ^a	17.8 ^b	9.1	14.9
Proline (µg/g, FW)	55.1	1866.1	52.6	1719.4	49.9	1958.2
Stomatal conductance (mmol m ⁻² s ⁻¹)	300.4 ^b	72.5 ^a	120.7	18.5	138.6	12.4
PhiPS2	0.64	0.66	0.66	0.68	0.6	0.65
NDVI	0.58	0.59	0.57	0.55	0.55	0.53
MCAR1	0.70 ^a	0.76 ^b	0.72	0.7	0.65	0.66
Lic2	0.86	0.86	0.85 ^a	0.94 ^b	0.99	1.05
ARI2	-0.1	-0.11	-0.07	-0.14	-0.18	-0.22
CRI2	1.86 ^b	1.63 ^a	1.78 ^b	1.12 ^a	1.07 ^b	0.52 ^a

PhiPS2 = photosystem II efficiency; NDVI = normalized difference vegetation index; Lic2 = Lichtenthaler Index; CRI2 = carotenoid reflectance index 2; MCAR1 1 = modified chlorophyll absorption reflectance index; ARI2 = anthocyanin reflectance index 2. The superscript letters following the mean values within the same status group for the same trait indicate the significance of the treatment effect within the same status group at $p < 0.05$ by emmeans pairwise comparison.

Table S5: Association between the stem elongation and anthesis stages for various physiological and biochemical traits averaged across 17 genotypes of *Triticum*.

Traits	Adjusted R-squared	P-value
Stomatal conductance (mmol m ⁻² s ⁻¹)	0.1717	<.0001
Relative water content (%)	0.4021	<.0001
Malondialdehyde (nmol/ml/g, FW)	0.4208	<.0001
Proline (µg/g, FW)	0.2591	<.0001
Photosystem II efficiency	0.008718	0.1992
Normalized difference vegetation index	0.02269	0.09909
Lichtenthaler index 2	-0.01194	0.7631
Anthocyanin reflectance index 2	-0.009859	0.6197
Modified chlorophyll absorption reflectance index2	0.000406	0.3131
Carotenoid reflectance index 2	-0.0004716	0.3294

Appendix III: Supplementary materials from Pantha et al. (2025),

Chapter 4

Pantha, S., Kilian, B., Özkan, H., Farooq M., Zeibig, F., Frei, M., 2025. A comparative analysis of drought tolerance mechanisms in *Triticum araraticum* Jakubz. and modern bread wheat (*Triticum aestivum* L.) cultivars

Journal of Agronomy and Crop Science (under review)

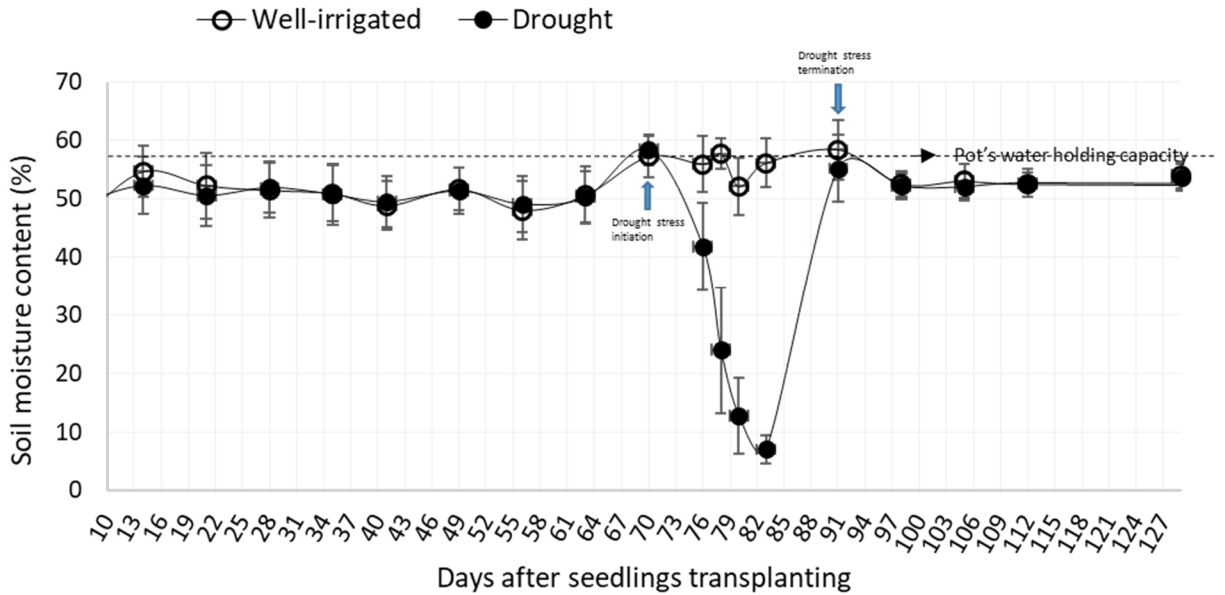


Fig. S1: Average soil moisture content under well-watered and drought treatments measured on different days after wheat seedlings were transplanted in pots under greenhouse conditions. Open and closed circles respectively denote well-watered and drought treatments. Error bars on the circles indicate mean \pm standard errors (n=8). The upward arrow denotes the time of drought initiation, while the downward arrow denotes the time of drought termination. The straight dotted line shows the average soil water-holding capacity of the pots.

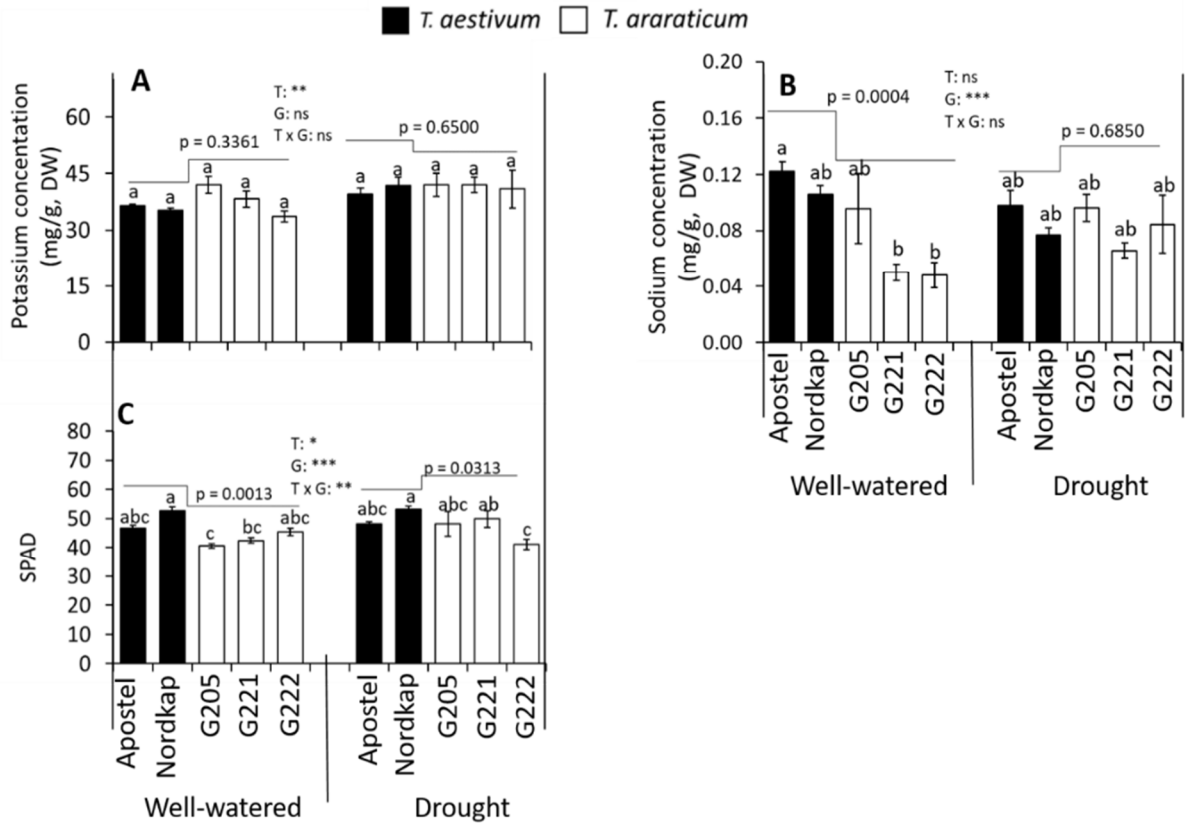


Fig. S2: Chlorophyll content and ion accumulation in different *Triticum* genotypes grown under well-watered and drought conditions. Potassium concentration (A) sodium concentration (B), and SPAD values (C). Vertical lines on the bars represent standard errors (n = 4). The letters above the bars respectively indicate significant differences across treatment by genotype interactions, as determined through pairwise comparisons using Tukey's test. Black and white bars respectively represent modern cultivars of *T. aestivum*, and genotypes of *T. araraticum*. The p-value shown by the elbow connector above the bars represent a comparison of the mean between the two species within the same treatment. A two-way ANOVA was performed to analyze the effects of treatment (T), genotype (G), and their interaction (T × G). ANOVA significance levels were: *p < 0.05, **p < 0.01; ***p < 0.001; and ns = not significant.

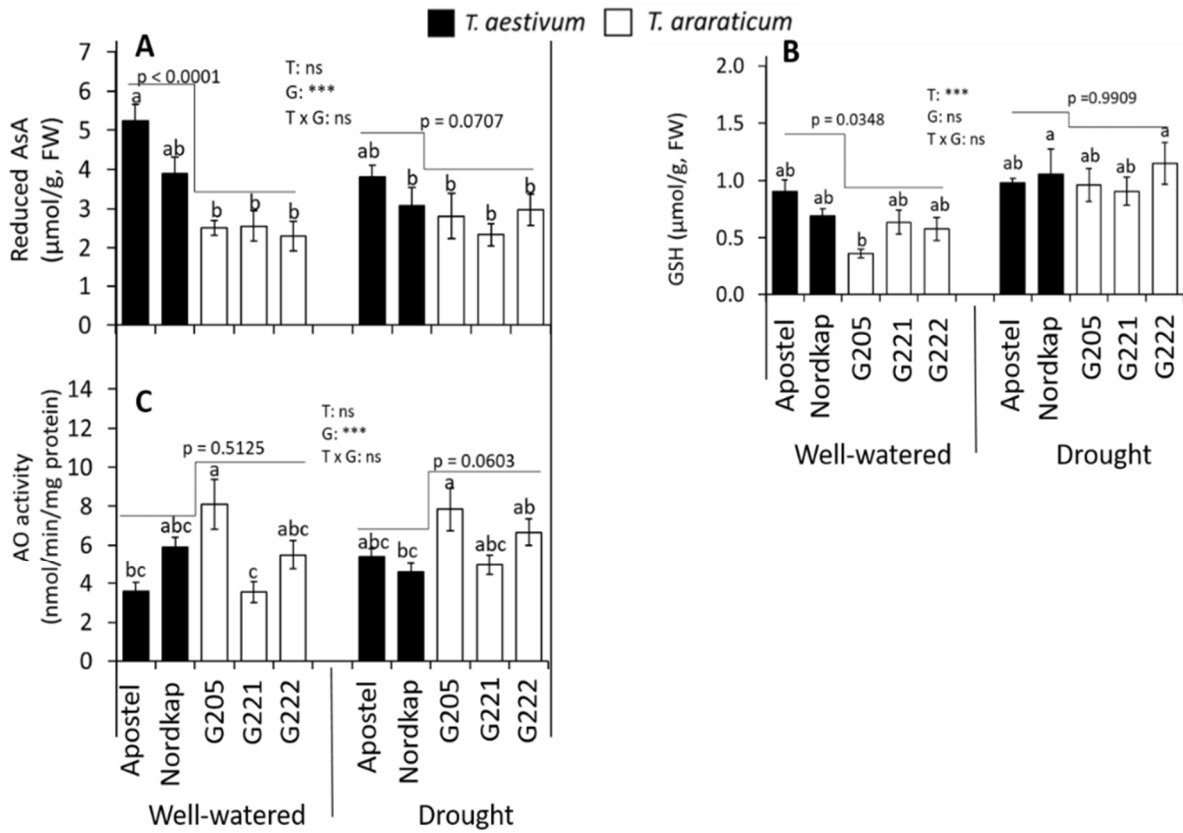


Fig. S3: Antioxidant levels in the leaves of different *Triticum* genotypes under well-watered and drought treatments: reduced ascorbate (A) reduced glutathione (B), and ascorbate oxidase (C). Vertical lines on the bars indicate standard errors (n = 4). The letters above the bars indicate significant differences across treatment by genotype interactions, as determined through pairwise comparisons using Tukey's test. Black and white bars respectively denote modern cultivars of *T. aestivum*, and genotypes of *T. araraticum*. The p-values shown by the elbow connectors above the bars represent a comparison of mean values between the two species within the same treatment. Treatment (T), genotype (G), and the genotype by treatment interaction (T × G) effects were tested with a two-way ANOVA. ANOVA significance levels were: *p < 0.05; **p < 0.01, ***p < 0.001; and ns = not significant.

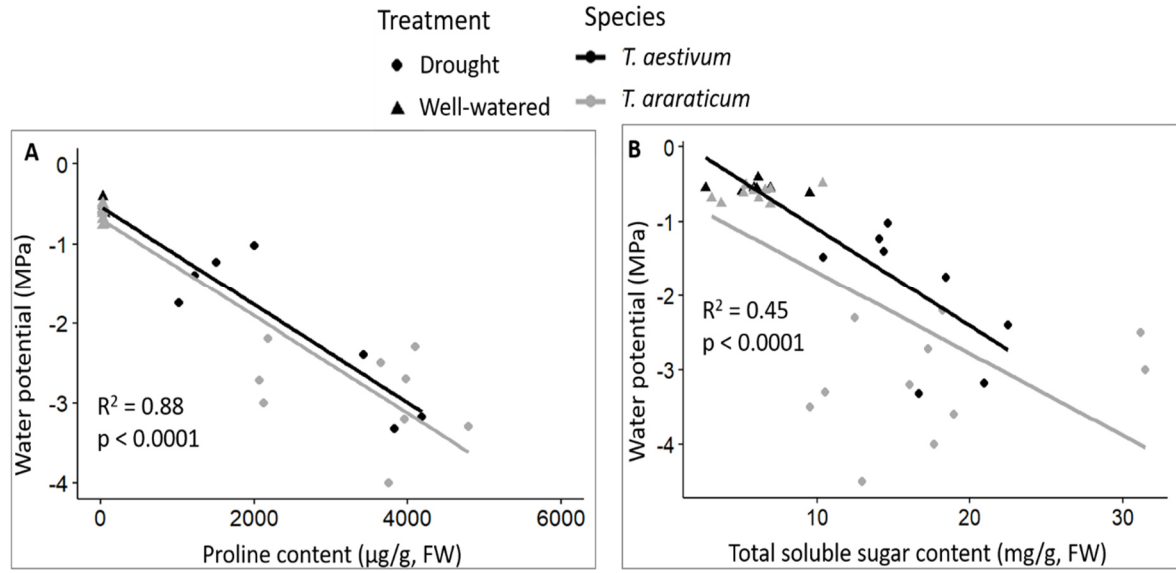


Fig. S4: The relationship between proline content and water potential (A), and between total soluble sugar content and water potential (B).

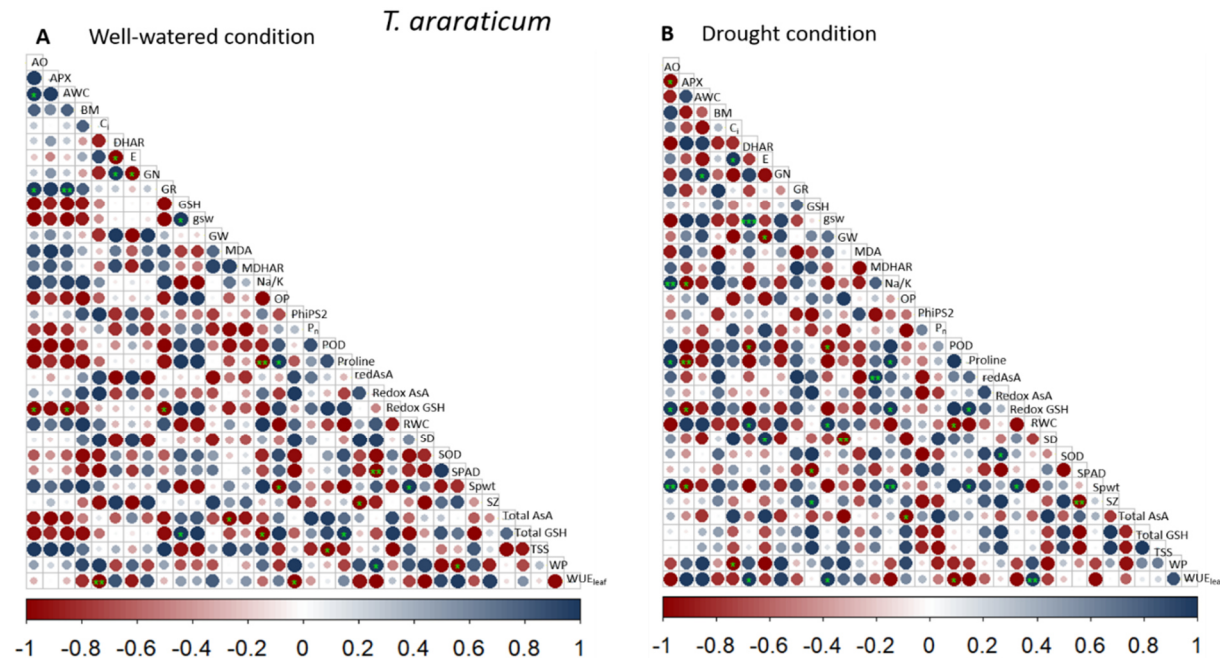


Fig. S5: Correlation plots showing the correlation coefficients for various physiological and biochemical parameters in *T. araraticum* under well-watered (A) and drought (B) treatments. ***, **, and * indicate significant correlations at $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. AO = ascorbate oxidase, APX = ascorbate peroxidase, AsA = ascorbate, AWC = absolute water content, BM = above-ground biomass per plant, C_i = internal CO_2 concentration, DHAR = dehydroascorbate reductase, E = transpiration rate, GN = number of grains per spike, gsw = stomatal conductance, GR = glutathione reductase, GSH = glutathione, GW = grain weight per spike, MDA = malondialdehyde, MDHAR = monodehydroascorbate reductase, Na/K = ratio of sodium to potassium concentration, OP = osmotic potential, PhiPS2 = photosystem II efficiency, P_n = net photosynthetic rate, POD = guaiacol peroxidase, redAsA = reduced ascorbate, RWC = relative water content, SD = stomata density, SOD = superoxide dismutase, Spwt = spike weight per plant, SZ = stomata length, TSS = total soluble sugar content, WP = leaf water potential, WUE_{leaf} = instantaneous photosynthetic water use efficiency.

	Genotype	Genetic group	Genome	Country of origin and seed collection site	Days to heading				Overall mean	Table S1: Days to
					Treatments		Mean			
					Drought	Well-watered	Drought	Well-watered		
<i>T. aestivum</i> L.	Apostel	Modern cultivar	BAD	I.G. Pflanzenzucht, Germany; SAATEN UNION,	169.1	170.0	169.4	170.5	170	to
	Nordkap			Germany; Iraq: 33.2 km west from Rowanduz toward Shaqlawa;	169.6	171.0				
	G205			Iraq: 4.4 km northwest from Amadiyah and Mazorka Gorge;	164.3	165.3				
<i>T. araraticum</i> Jakubz.	G221	Wild	GA ^t	Iraq: 19.3 km south from Sulaymaniyah toward Qara Dagh	163.1	161.1	162.2	161.8	162	
	G222			159.1	159.0					

heading in two different wheat species, along with the genotype details.

Acknowledgments

First of all, I would like to express my deep gratitude to my first supervisor Prof. Dr. Michael Frei for providing me the opportunity to do my PhD thesis under his guidance. I consider myself incredibly fortunate to have a supervisor like him, who is always positive and encouraging. His support and scientific freedom enabled me to conduct the research and overcome challenges. I am always impressed by his open-minded perspective. Furthermore, I truly appreciate his prompt responses, whether it is for official matter (e.g. manuscript or report feedback) or other personal matters. Thank you, Prof. Frei, for providing a family-friendly working environment that enabled me to manage my stay in Germany with my child, while conducting this PhD research. Additionally, my sincere thanks goes to my second supervisor Prof. Dr. John Clifton-Brown for his suggestions during my PhD.

I also wish to express my sincere thanks to Dr. Benjamin Kilian for his continuous scientific support during this PhD journey. He provided valuable feedback to the manuscripts, which enabled us to publish in reputable journals. I would also like to thank Prof. Dr. Hakan Özkan and Prof. Dr. Muhammad Farooq for providing scientific support. Additionally, I would like to thank the BOLD project.

I also want to thank all my colleagues at the Department for Agronomy and Crop Physiology. I would like to thank Dr. Frederike Zeibig. We worked in good collaboration together to explore wild wheats. My sincere thanks goes to Liane Renno for her continuous support during field and greenhouse experiments, and lab works. I will never forget the fun we had together while working. I would further like to express my thanks to Erika Schick for her assistance during my thesis research; she was always ready whenever needed. Moreover, I would like to thank Dr. Yavar Vaziritabar, Dr. Linbo Wu, Dr. Andriale Wairich, Dr. Johanna Krippner, Dr. Yanru Feng, Dr. Sawitree Autarmat, Denise Schudt, Christine Kessler-Turturici, Dr. Muhammad Shahedul Alam, Lea Schmitt, Lisa Marie Moosmann, Mishel Risilia, Emily Siegward, Moritz Jehner, Lukas Engelbach, Heiko Dettweiler and Mohannad Alobid for their cooperation and help during my research works. I also like to express my special thanks to Ambika Pandey for her invaluable assistance not only in research, but also for taking care of my child during the first year of my PhD research, when I really had tough time managing far away field experiment in Gross-Gerau. I also express my thanks to Mario Tolksdorf and his team at the Gross-Gerau field station for their support during the field work. I would also like to thank colleagues from the Plant Nutrition Department, Dr. Tabea Selzer and Tanja Ruppert for their support.

I am very grateful to my beloved family. First I would like to thank my parents, who are always supportive and encouraging. My father is my role model for hard work and honesty, and my mother is always supportive, with a pure heart and innocence. I am immensely thankful to my loving and supportive husband Resham for everything he has provided to me for pursuing my academic and scientific ambitions. His understanding and encouragement, despite the distance my PhD has put between us, have always been a great source of strength to me. I would like to thank my son Himalaya for his unwavering patience and understanding, when I had busy schedule. You are my greatest companion in this PhD journey. I am really proud of you for being such a kind and cooperative child. You are my strength in hard times, and I am truly blessed to have you in my life. I am also really thankful to have such amazing siblings, Samjhana, Mira, Sarita, Kailash and Kalyan in my life. Your care and chit-chat, even virtually, always make me feel relieved in stressful time.

I would also like to thank to the German Academic Exchange Service (DAAD) for providing PhD scholarship and thanks to the Nepal Agricultural Research Council for providing study leave.

ki@JLU was used to improve the legibility of this dissertation by checking grammar and spelling, and enhancing readability.

Declaration

I declare that the doctoral thesis here submitted is entirely my own work, written without any unauthorised help by a third party and solely with the assistance referred to in the thesis. I have indicated in the text those texts that have been quoted from already published sources, either verbatim or by analogy and all statements based on verbally conveyed information. During the research carried out by me and referred to in the doctoral thesis, I have at all times followed the principles of good scholarly practice as defined in the “Statute of the Justus Liebig University Giessen for Ensuring of Good Academic Practice”.

Giessen, _____

Sumitra Pantha