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Exploring the genetic toolbox for climate-resilient spring wheat

Drought impact on yield, breadmaking quality, nutritional value and toxicity

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Cover: A field under drought condition, wheat plants examined in a greenhouse, and mature wheat plants grown under blue sky. Illustration by Yuzhou Lan.

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Abstract

Climate change is posing challenges to wheat production worldwide through increasing frequency of drought events. In southern Sweden, summer drought typically coincides closely with the reproductive growth stages of wheat, the most critical period for final yield. This thesis characterised the drought tolerance of wheat from a broad set of genetic materials, based on traits relating to yield, breadmaking quality, nutritional value and toxicity. The possibility of using biostimulants to enhance grain yield of wheat under drought was also explored.

Late drought was found to restrict grain yield to a greater extent than early drought. Old Swedish cultivars and wheat lines carrying chromosome 1R had significantly longer roots during early stage, enabling better access to water. However, there was a strong connection between this long-root trait of old and 1R genotypes and elevated grain cadmium content, posing a possible risk to food security. There was generally a negative correlation between protein concentration and grain yield. However, high grain protein content and high grain yield were observed in some wheat lines carrying chromosome 3R, indicating opportunities for breeding high-protein varieties without compromising yield. Lines with 3R possessed advantageous attributes in terms of minerals, with high zinc and iron concentrations. High grain selenium concentration was observed in lines carrying chromosome 1R, suggesting unique nutritional values of those wheat-rye introgression lines compared with modern wheat. Use of biostimulants (chitosan, hydrolysed wheat gluten and potato proteins) had positive effects on grain yield in wheat grown under drought.

The new information provided in this thesis on utilising genetic resources from distant relatives of wheat and ancient wheat can be applied to improve the environmental resilience of modern wheat, while applying biostimulating compounds can be a fast, cost-effective way of sustaining wheat growth during drought.

Keywords: Wheat, drought, grain yield, breadmaking quality, nutritional value, heavy metal content, biostimulant

Utforskning av genetisk verktygslåda för klimatåligt vårvete

Torkans inverkan på skörd, brödtillverkningskvalitet, näringsvärde och toxicitet

Sammanfattning

Klimatförändringar utmanar veteproduktionen över hela världen genom att öka frekvensen av torkhändelser. I södra Sverige sammanfaller sommartorka vanligtvis nära de reproduktiva tillväxtstadierna för vete, den mest kritiska perioden för slutskörd. Denna avhandling karakteriserade vetens torktålighet med hjälp av en bred uppsättning genetiska material, baserat på egenskaper relaterade till skörd brödkvalitet, näringsvärde och toxicitet. Möjligheten att använda biostimulanter för att förbättra vetens kornskörd under torka utforskades också.

Sen torka visade sig begränsa spannmålslörd i större utsträckning än tidig torka. Gamla svenska sorter och vetelinjer som bar kromosom 1R hade signifikant längre rötter under tidiga stadier, vilket möjliggjorde bättre tillgång till vatten. Det fanns emellertid en stark koppling mellan denna långa rotegenskap hos gamla och 1R-genotyper och förhöjt kadmiuminnehåll i kornet, vilket utgör en möjlig risk för livsmedelssäkerheten. Det fanns generellt en negativ korrelation mellan proteinhalt och kornkörd. Dock observerades hög kornproteinhalt och hög kornskörd hos vissa vetelinjer som bar kromosom 3R, vilket indikerar möjligheter att förädla högproteinsorter utan att påverka skörden. Linjer med 3R hade fördelaktiga egenskaper vad gäller mineraler, med höga zink- och järnkoncentrationer, medan kornets selenhalt var hög hos linjer som bar kromosom 1R, vilket antyder unika näringsvärden hos dessa vete-råg introgressionslinjer jämfört med modern vete. Användningen av biostimulanter (kitosan, hydrolyserat vetegluten och potatisproteiner) hade positiva effekter på kornskörden hos vete som odlades under torka.

Den nya informationen som presenteras i denna avhandling om att använda genetiska resurser från avlägsna släktingar till vete och antika vetesorter kan tillämpas för att förbättra den miljömässiga motståndskraften hos modern vete, medan användningen av bioaktiva föreningar kan vara ett snabbt, kostnadseffektivt sätt att upprätthålla vetens tillväxt under torka.

Nyckelord: Vete, torka, kornskörd, brödkvalitet, näringsvärde, tungmetallhalt, biostimulant

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Lan, Y., Chawade, A., Kuktaite, R. & Johansson, E. (2022). Climate change impact on wheat performance - effects on vigour, plant traits and yield from early and late drought stress in diverse lines. *International Journal of Molecular Sciences* 23(6), 3333. DOI: 10.3390/ijms23063333.

II. Lan, Y., Kuktaite, R., Chawade, A. & Johansson, E. (2023). Diverse wheat lines - to mitigate the effect of drought on end-use quality. *Frontiers in Food Science and Technology* 3, 1163412. DOI: 10.3389/frfst.2023.1163412.

III. Lan, Y., Kuktaite, R., Chawade, A. & Johansson, E. (2024). Chasing high and stable wheat grain mineral content: Mining diverse spring genotypes under induced drought stress. *PLoS ONE* 19(2): e0298350. DOI: 10.1371/journal.pone.0298350.

IV. Lan, Y., Kuktaite, R., Chawade, A. & Johansson, E. (2024). Combating heavy metals in wheat grains under drought – is alien or ancient germplasm a solution to secure food and health? *Journal of Agriculture and Food Research* 101118. DOI: 10.1016/j.jafr.2024.101118.

V. Lan, Y., Jolayemi, O.L., Chawade, A. & Johansson, E. (2024). Organic compounds sustaining wheat growth under drought – biostimulants applied by seed priming and foliar spraying (manuscript).

Papers I-IV are reproduced with the permission of the publishers.

Other Publications not included in the thesis

Lan, Y., Burca, G., Yong, J. W. H., Johansson, E., & Kuktaite, R. (2024). New Insights into the Bio-Chemical Changes in Wheat Induced by Cd and Drought: What Can We Learn on Cd Stress Using Neutron Imaging? *Plants*, 13(4), 554. DOI: 10.3390/plants13040554.

Johansson, E., Kuktaite, R., Labuschagne, M., Lama, S., Lan, Y., Nakimbugwe, D., ... & Vazquez, D. (2023). Adaptation to abiotic stress factors and their effects on cereal and pseudocereal grain quality. In Developing Sustainable and Health Promoting Cereals and Pseudocereals (pp. 339-358). Academic Press. DOI: 10.1016/B978-0-323-90566-4.00001-1.

1. Introduction

Under the macro trend of climate change, global temperature has increased over decades and is predicted to climb even faster in the future (Allen *et al*., 2019). As a result, drought events of increasing duration and intensity are occurring more frequently worldwide. Drought stress has therefore become the major yield-limiting abiotic factor (Lambers *et al*., 2008). Severe yield losses caused by drought have been recorded in different regions of the world. For instance, a drought-induced decrease of 42% in cereal production, accompanied by an economic loss of 2500 million Euros, was reported in Spain in 2005 (European Commission, 2007). This compromises crop yield and prevents the increase in world food production required to meet demand from the rapidly growing global population.

Wheat (*Triticum aestivum*) is currently the world's most widely grown crop (221 million hectares harvested in 2021; https://www.fao.org/faostat/en/#compare, accessed 25 July 2023), contributing around 20% of all calories and proteins in the human diet (Shiferaw *et al*., 2013). However, even in a high-latitude country such as Sweden, with much cooler weather than southern European countries, the wheat crop can still be hit by heat and drought during the summer. Swedish summer typically coincides with the reproductive growth stages of wheat, which is the most water-sensitive period of the entire life cycle for yield formation. Therefore, lack of rainfall during summer can pose a major threat to wheat productivity in Sweden. In 2018, when summer heat was combined with severe drought, wheat production level in Sweden fell to its lowest since 1995 (https://www.fao.org/faostat/en/#data/QCL/visualize; accessed 31 July 2023). Such summer drought is projected to occur more frequently in Sweden in the future (Grusson *et al*., 2021). Thus, there is an urgent need to breed wheat lines with better resilience to drought.

Besides compromising yield, drought also affects grain quality traits such as protein content and quality and mineral concentrations. Around 80% of wheat protein consists of gluten proteins, which has the attribute of forming large polymeric protein networks during processing (Johansson *et al*., 2013). Gluten content and composition therefore largely determines the end-use quality of wheat flour, and especially the bread-making quality. The content of different micronutrient minerals in wheat grain determines its nutritional value. Although required in small amounts, micronutrients play a critical role in almost all life activities of the human body, *e.g*. the strength of the skeletal structure, absorption of vitamins and digestion-related enzyme activities (Gupta and Gupta, 2014). Inorganic mineral nutrients are transferred to organisms via the soil-plant-animal/human pathway. Thus, selecting and developing wheat lines with high and stable mineral content is of critical importance in preventing undernourishment in a future varying climate.

The interaction between wheat plants and drought stress is complicated by the characteristics of diverse wheat genotypes and the timing of droughts. All the studies on which this thesis is based (Papers I-V) were designed to include early and late drought, so that the impacts of these two types of drought could be explicitly evaluated. Investigations on these drought impact on wheat genotypes with different genetic backgrounds focused on the aspects of (i) morphological, physiological and yield traits (Paper I), (ii) protein quality traits (Paper II), (iii) grain mineral and heavy metal concentrations (Paper III-V).

2. Background

2.1 Drought events under climate change

Drought occurs when a certain region lacks water supply. In meteorological and hydrological terms, lack of water is mainly caused by a reduction in precipitation, combined with rapid evaporative moisture loss induced by warming weather. In agricultural terms, lack of water refers to water shortage in soil layers that the roots of crops can access, causing drought stress (Mannocchi *et al.*, 2005). Multiple cases of unprecedented drought-induced crop failure or severe yield reduction have been documented worldwide during the past five decades. Over that period, the average cereal yield reduction caused by drought and heat stress in Europe has tripled, with specifically drought-related yield reductions increasing at a rate of 3% per year (Brás *et al*., 2021). In 2018, northern Europe experienced multiple crop failures associated with unusually low precipitation and high temperatures (Beillouin *et al*., 2020). In 2012, the USA experienced one of its worst droughts in 80 years. In 2006, severe drought was recorded in south-west China, resulting in crop failure on over 311,300 ha (Li *et al*., 2011; Lin *et al*., 2015).

Sweden is not an exception to global warming. As shown in Figure 1, the Swedish mean annual temperature deviation is shifting towards a larger positive value, with a faster temperature increase projected in the future, and this warming weather is already impacting wheat cultivation in Sweden. In the unusually hot, dry summer of 2018, southern Sweden as a whole had temperatures 2-4 °C higher than average and temperature records were broken in the southern provinces of Götaland and Svealand (SMHI, 2018).

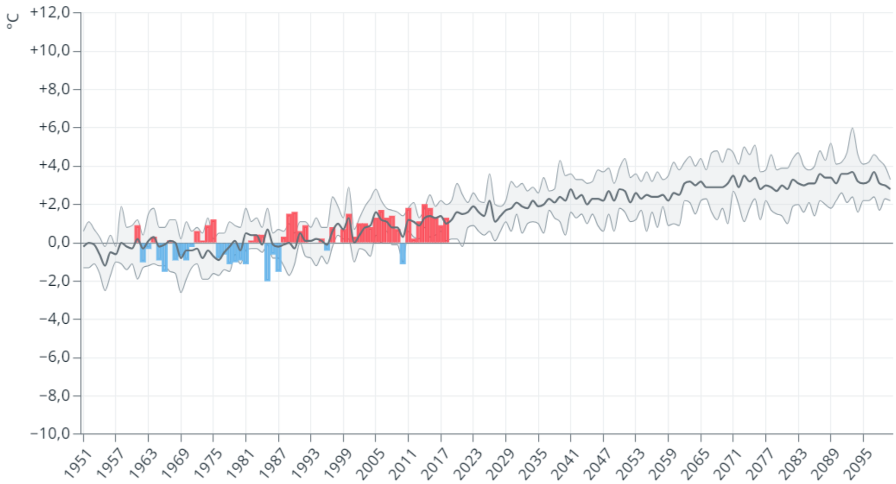

Figure 1. Change in mean annual temperature (°C) in Sweden during the period 1951-2023 and projected change in the period to 2100, compared with the reference period 1971-2000. Data source: Swedish Meteorological and Hydrological Institute (SMHI).

According to the Food and Agriculture Organization of the United Nations (FAO), Swedish wheat productivity suffered a 21% reduction in area harvested, a 38% reduction in yield and a 51% reduction in total production in 2018 compared with the previous year (Figure 2). These severe reductions were strongly associated with the hot, dry summer recorded in 2018, providing a clear indication that climate change is an ongoing reality, rather than a prospective future threat, and that drought is one of the climate change outcomes posing substantial challenges to food security. In order to develop and update strategies for coping with varying current and future climate, studies are urgently needed on multiple aspects of the interactions between drought and wheat.

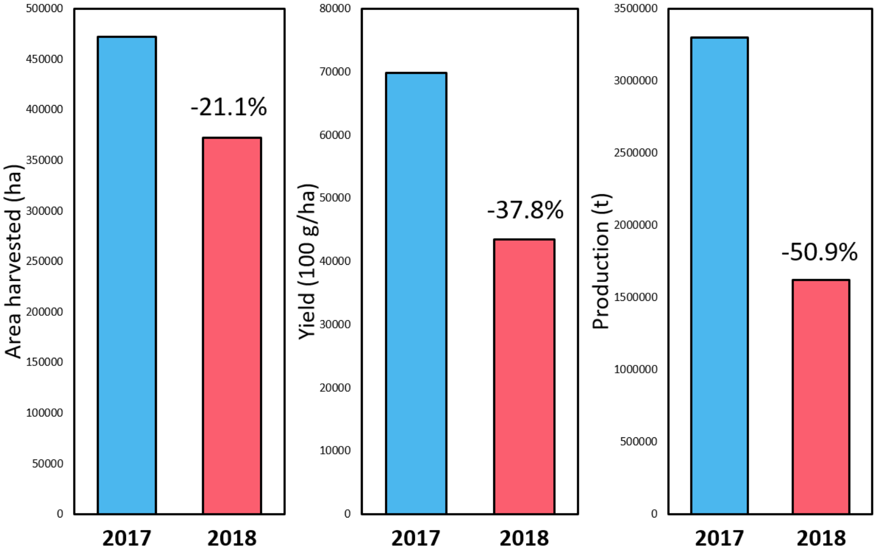

Figure 2. Aspects of Swedish wheat production in the drought year 2018 compared with the year 2017. Data source: Food and Agriculture Organization of the United Nations (FAO).

2.2 Wheat from diverse genetic sources

Wheat genotypes bred from different backgrounds or evolved under different environments form the crucial germplasm pool encompassing genes that can be utilised for various breeding goals, *e.g.* yield increase, quality improvement, disease resistance and climate resilience.

2.2.1 Modern breeding lines

Wheat breeding research began at different time points across the world and therefore the threshold applied to separate modern and old wheat lines differs (Mefleh *et al*., 2019; Shewry e*t al*., 2016). Although the true breakpoint is still unclear, the term ‘modern wheat’ mainly refers to wheat developed in intensive breeding cycles and subjected to years of selection to improve yield performance. One good example of the yield increase brought about by breeding techniques is the successful development of semi-dwarf cultivars achieved by the introduction of *Rht-1* dwarf genes into wheat in the late 20th Century (Hedden, 2003). With reduced height, wheat plants have the characteristic of shorter and stiffer straw, which imparts higher nitrogen use efficiency and lodging resistance, resulting in an improvement in harvest index (Mefleh *et al.*, 2019). Modern wheat cultivars have a strong common feature of high yield performance. However, introduction of the *Rht-1* loci also results in insensitivity of the plants to gibberellic acid, which inhibits other gibberellic acid-dependent developmental processes such as root growth and shoot elongation (Bai *et al.*, 2013). Thus, in addition to the high yield performance and largely unified small shoot size, modern wheat lines also share the feature of a small root system, which can be a disadvantageous trait under increasingly dry growing season conditions (Würschum *et al.*, 2017).

2.2.2 Old Swedish cultivars

Thanks to their great genetic diversity, old Swedish wheat cultivars are known to adapt well to varying and low-input environments (Diederichsen *et al.*, 2013). Compared with modern wheat, these old cultivars require less nutrient input and pest/weed control, and the grains are rich in protein and mineral content, giving high nutritional value. Some primitive wheat genotypes have been found to be specifically suitable for organic farming systems in Sweden, due to their high content of minerals (Hussain *et al.*, 2010) and other health-promoting compounds, *e.g.* tocopherols and tocotrienols (Hussain *et al.*, 2012b). These advantageous traits are likely due to the generally larger root system of old wheat compared with modern wheat lines (Bektas et al., 2016; Bektas and Waines, 2018). The vigorous root system of old cultivars is also known to benefit plant growth under water-limited conditions (Palta *et al.*, 2011). From the perspective of disease resistance, the natural diversity, together with efforts to incorporate resistance genes in Swedish wheat breeding during the late 20th Century, have contributed to a wide resistance spectrum in Swedish old wheat (Diederichsen *et al.*, 2013; Olsson, 1970). Thus, despite their lower yield performance than modern wheat, old cultivars are of great relevance in coping with stressful biotic and abiotic factors owing to their high adaptability.

2.2.3 Wheat-rye introgression lines

Chromosomes 1R, 2R, 3R, 5R and 6R in rye (*Secale cereale* L.) have been found to be essentially homologous with wheat chromosome groups 1, 2, 3, 5 and 6, thereby allowing homologous pairing between chromosomes from these two species (Bauer *et al*., 2017). Further, the allopolyploid nature of common bread wheat makes it more compatible with modifications to the genome. While crosses with old cultivars possessing ancient genes can compensate for the diversity loss in modern wheat, crossing wheat with its distant relatives, *e.g.* rye, equips it with genes that have never previously existed in the wheat genome. Rye is the most exploited alien genetic donor in work to expand the gene pool of wheat and is known to carry genes contributing to a range of superior traits, *e.g*. resistance to diseases (Mains, 1923; Vendelbo *et al*., 2021) and tolerance to harsh climates (Geiger and Miedaner, 2009). Successful transfer of chromosome 1RS into wheat-rye introgression lines has been reported to confer resistance to stem rust, stripe rust, leaf rust and powdery mildew (Purnhauser *et al*., 2011; Ren *et al*., 2012). Improvements in root traits have also been achieved by 1R and 1RS introgressions (Howell *et al*., 2019; Liu *et al*., 2020), and can likely contribute to yield performance and drought tolerance. Thus, utilising rye as alien germplasm to broaden the spectrum of wheat adaptation to climate change is of significant interest.

2.3 Drought impacts on wheat

2.3.1 Morphological traits

In a broad sense, grain yield components, *e.g*. spike traits, number of grains per spike and grain weight, can be categorised as part of wheat morphology. However, the morphological traits discussed in this thesis refer to plant size- and shape-related traits that only involve characteristics of roots, stems and leaves, while the spikes and grains are discussed separately due to their prominent importance for final yield. The most directly size-related traits in different parts of a wheat plant are root length, plant height and leaf area. The root system forms the connection between plant shoots and soil and senses at first-hand any changes in soil moisture. It therefore responds more directly than the above-ground plant parts. Under drought, wheat plants have been found to show reduced total root biomass and deeper root allocation (Friedli *et al*., 2019; Zhang *et al*., 2004), giving the root system better ability to access water potentially stored in deep soil layers. In above-ground plant parts, drought stress reduces plant height by inhibiting photosynthesis and nutrient translocation in wheat, especially if the stress occurs during the stem elongation stage (Sarto *et al.*, 2017). The restriction in photosynthesis is mainly due to abscisic acid-regulated leaf stomatal closure triggered under drought as a means to prevent or slow down further water loss (Saradadevi *et al.*, 2017). Significantly decreased area of leaves has also been observed in wheat under drought (Ahmad et al., 2018), while the ability of flag leaves to remain green has been found to be associated with the yield-maintaining ability of wheat under drought (Foulkes *et al.*, 2007).

2.3.2 Yield components

Humans grow wheat mainly for food purposes, making grain yield the most relevant trait to meet global food demand and closely connected to the second of the 17 Sustainable Development Goals established by the United Nations, *i.e.* zero hunger (Economic and Affairs, 2022). However, wheat yield varies depending on various factors, *e.g.* climate, disease, soil fertility, agricultural practices, wheat variety itself and the increasing drought events induced by climate change, which have become the major yield-restricting environmental factor in wheat cultivation (Chaudhry and Sidhu, 2022). Severe yield reductions in wheat have been documented in different studies assessing different types of drought. Whole-life-circle drought has been shown to induce an 84% yield reduction in wheat plants (Wan *et al.*, 2022). Prolonged drought during the most critical periods, anthesis and grain filling, can cause a 58-92% yield reduction (Farooq *et al.*, 2014), while post-anthesis drought can reduce grain yield by 26% (Wan *et al.*, 2022).

In addition to grain yield, yield components also consist of other spike and grain traits, *e.g.* number of spikes, spike length, number of grains and grain size. One study found that drought can induce a 21% reduction in number of spikes per plot (Pour-Aboughadareh *et al.*, 2020). In another study, a 12-59% reduction in number of grains per spike was observed among drought-treated modern wheat and landraces compared with a control (Vahamidis *et al.*, 2019). Thousand-grain weight is the most commonly used agronomic parameter to describe grain size, and it can be severely decreased (by up to 45%) under drought stress (Pour-Aboughadareh *et al.*, 2020).

2.3.3 Bread-making quality

Due to their broad functionality spectrum, wheat grains can be processed into many food products, *e.g.* bread, pasta, noodles and biscuits (Johansson *et al.*, 2013). Among these, bread is often considered the highest-volume and most widespread food product and serves as a staple in many diets around the world. The breadmaking quality is largely determined by the protein quality of the flour (Gupta *et al*., 1993). Wheat protein quality can be represented by the molecular size distribution of gluten proteins, *i.e*. gliadins and glutenins, which together make up 85% of grain proteins (Johansson *et al*., 2020; Markgren *et al*., 2020). Gliadins and glutenins differ in terms of their solubility and are monomeric and polymeric, respectively, in their native state, with their conformation stabilised by disulphide and inter-chain hydrogen bonds (Belton, 1999; Keck *et al*., 1995). However, during post-harvest mechanical or chemical processing, *e.g*. grain milling, dough mixing, pressing, heated drying and protein extraction, the intra- or inter-molecular disulphide bonds of these gluten proteins can be broken and reformed into new inter-molecular cross-links that bind surrounding glutens together, forming new large protein polymers (Markgren *et al*., 2022; Rasheed *et al*., 2015). This unique characteristic is referred to as the polymerisation behaviour of gluten proteins.

Environmental stresses during wheat growth, *e.g*. heat and drought, have been shown to impact polymerisation of the gluten proteins, and also to shorten the life cycle of the plant under adverse conditions (Johansson *et al*., 2013; Labuschagne *et al*., 2021). More specifically, drought has been found to cause higher concentrations of unextractable polymeric proteins, owing to drought-induced grain water loss, whereas high water content in grains is known to weaken the electrostatic and hydrogen bonds in polymeric proteins (Johansson *et al*., 2008; Leiva *et al*., 2021). In a study where soil moisture content was lowered to 45% after the flowering stage of wheat, significantly increased concentrations of total grain protein, gliadin and glutenin and higher glutenin/gliadin ratio were observed (Zhao *et al*., 2009a).

2.3.4 Mineral composition

Most minerals are only required in small amounts by the human body, but play a crucial role in maintaining balanced nutritional status (Gupta and Gupta, 2014). Through their high daily consumption, wheat products serve as an important dietary source of mineral intake for humans, and therefore the composition and concentrations of different minerals in wheat grains largely determine the nutritional value. Zinc (Zn) and iron (Fe) are the two main health-related minerals in wheat grain. Low zinc intake is known to cause a series of health issues in humans, *e.g*. a weak immune system, liver disease and diarrhoea (Prasad, 2009), while iron deficiency is the major cause of anaemia, a serious global health problem particularly affecting children and women (Camaschella, 2015; Paesano *et al*., 2010). Selenium (Se) is another vital trace nutrient that is gaining increasing attention due to its potential benefits in human health, *e.g*. in cancer prevention (Clark *et al*., 1996) and immunity enhancement (Broome *et al*., 2004). Wheat grains also contain other micronutrients, *e.g*. calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), phosphorus (P) and sulphur (S), that are necessary in order for the human body to maintain good health status (Martínez-Ballesta *et al*., 2010).

Minerals in the soil are absorbed by roots mainly as ions, with water serving as the transport medium during this process. Thus, impacts of drought on mineral uptake by plants are determined by two major factors that interact with each other, *i.e*. soil physiochemical conditions and root properties (Amtmann and Blatt, 2009). Reductions in soil moisture content directly affect nutrient mobility in the soil, which can be measured as ion diffusion rate, and this restricted mobility leads to a decrease in available nutrient transport from soil pores to root surface (Viets and Kozlowski, 1972). Lack of water and nutrients in turn inhibits vegetative growth of the whole plant, including root growth. However, plants bred or evolved from different genetic sources possess different intrinsic root attributes, among which soil-to-root absorption capacity, root-to-leaf translocation ability and root extension ability are the most relevant to mineral uptake under water deficit conditions (Viets and Kozlowski, 1972). Large variations in grain iron and zinc concentrations have been found among a set of bread wheat genotypes, with *e.g*. spelt, einkorn and emmer wheat genotypes exhibiting significantly higher accumulation of selenium than common bread wheat (Zhao *et al*., 2009b). Further, a clear trend of declining grain mineral content with ascending year of wheat genotype release has been reported in different studies (Fan *et al*., 2008; Zhao *et al*., 2009b).

2.3.5 Heavy metal content

Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) are toxic to both humans and ecological systems (animals and plants), and their presence in the environment has significantly increased due to years of large-scale industrial activities such as mining and smelting (Shallari *et al*., 1998). Heavy metals can accumulate in the human body, causing severe health issues with chronic exposure. One major pathway of heavy metals entering the human body is through intake of contaminated foods, *e.g.* wheat. The large-scale consumption of wheat makes it a risk source in this regard, as grains harvested from contaminated soils have been found to readily accumulate heavy metals (Abedi and Mojiri, 2020; Greger and Löfstedt, 2004; Jafarnejadi et al., 2011). Soils in Skåne, a province in southern Sweden, are reported to contain higher levels of cadmium than soils in other regions, thereby increasing the risk of elevated cadmium accumulation in grain (Eriksson and Söderström, 1996). Available information about the interaction between drought and heavy metal accumulation remains limited, but drought has generally been found to lower the cadmium content in durum wheat (Lan et al., 2024), *Ricinus* and *Brassica* species (Bauddh and Singh, 2012) castor bean (Shi *et al*., 2015) and peanuts (Liu *et al*., 2017). In contrast, a trend for elevated cadmium accumulation has been observed in wheat plants grown under drought (Abbas *et al*., 2018). Furthermore, heavy metal uptake is reported to differ widely between crop cultivars, suggesting both risk and opportunity (Hussain *et al*., 2012a; Yang *et al*., 2022). Thus, the interaction between drought and heavy metal accumulation is affected by a number of factors, such as plant species, growth stage, intensity of drought and severity of heavy metal stress. Under ongoing climate change, rigorous assessments of heavy metal accumulation in wheat grains are needed to prevent potential exposure.

2.4 Biostimulants and drought tolerance

Biostimulants are a set of substances that function to enhance plant growth in aspects such as nutrient uptake, nutrient efficiency and tolerance to abiotic stress (du Jardin, 2015). These substances derive from a wide range of sources, including plants, animals and microorganisms (Xu and Geelen, 2018). Thus, instead of developing drought-tolerant cultivars, which usually take years of field trials, biostimulants can be an effective alternative solution. Mitigation of drought stress by biostimulants has been observed in different plant species, *e.g.* sugarcane (Jacomassi *et al*., 2022), sweet basil (Taha *et al*., 2020) and tomato (Wang *et al*., 2022).

Depending on different sources and active ingredients, biostimulants can be divided into five groups: seaweed extracts, fulvic and humic acids, plant growth-promoting bacteria, protein-based biostimulants, and chitosan (du Jardin, 2015). Part of the work in this thesis focused on two of these categories, protein-based biostimulants and chitosan. Protein-based biostimulants are derived by hydrolysing side-streams of plant and animal raw materials with a high content of proteins (Moreno-Hernández *et al*., 2020). Chitosan is produced by removing the acetyl groups from chitin extracted from the shells of sea animals and insects (Shahrajabian *et al*., 2021). Thus a feature of protein-based biostimulants and chitosan is that their sources are usually agricultural by-products or food wastes, giving them advantages in terms of cost, accessibility, abundance and sustainability (Xu and Geelen, 2018). However, reports on the effect of these specific types of biostimulants in sustaining plant growth during drought are still lacking. There is therefore a need to explore possibilities of utilising those cheap and accessible compounds in efforts to combat yield losses associated with climate change.

3. Aims and Objectives

The main aims of this thesis were to evaluate the effect of drought stress on wheat, covering most of the aspects relevant to food security, *i.e*. grain yield, protein quality, nutritional value and toxicity, and to study the relationship between different drought-responding traits. The performance of different genotype groups in this regard was assessed in an attempt to identify superior germplasm for future breeding. Beyond wheat breeding efforts, the potential of using biostimulants to promote drought tolerance of wheat was explored. Specific objectives of the work in Papers I-V were to:

- Determine the effects of early and late drought on wheat grain yield, protein quality, mineral composition and heavy metal content (Papers I-IV).
- Evaluate wheat performance in terms of early vigour, morphology and yield, and relate those traits to different genetic backgrounds (Paper I).
- Search for wheat genotypes with both high grain yield and protein content, and analyse the stability of each genotype across control, early drought and late drought conditions (Paper II).
- Identify rye chromosomes from wheat-alien introgressions that may carry genes contributing to high and stable mineral content in wheat grain (Paper III).
- Assess the risk of excessive heavy metal accumulation in grains of alien and ancient wheat (Paper IV).
- Evaluate the drought tolerance of wheat lines treated with different biostimulants and compare the effectiveness of two application methods (seed priming, foliar spraying) (Paper V).

4. Methods

4.1 Wheat material resources

In Papers I and II, a total of 73 spring wheat genotypes were used, consisting of nine modern wheat breeding lines provided by the company Lantmännen, 14 old Swedish cultivars released between 1982 and 1990, and 50 wheat-alien introgression lines carrying rye chromosomes 1R, 1RS, 2R, 3R, 4R, 5R, 6R and *Leymus racemosus* chromosome N.

In Papers III and IV, 30 spring wheat genotypes were used, comprising five modern breeding lines, five old Swedish cultivars and 20 wheat-rye introgression lines with rye chromosomes 1R, 1RS, 2R and 3R.

In Paper V, one modern breeding line and one old Swedish cultivar were used.

4.2 Biotron and greenhouse trials

Wheat plants were grown in a biotron chamber at the Swedish University of Agricultural Sciences in Alnarp, Sweden, under strictly hourly regulated temperature and humidity based on a 10-year average climate dataset obtained from the Swedish Meteorological and Hydrological Institute (SMHI) (Papers I-IV). A greenhouse trial was specifically designed for Paper V, where plants were grown at 25-30 ℃ and 89-90% relative humidity. Biostimulants were applied by two methods, seed priming and foliar application. Seed priming was performed by soaking seeds in 25 mL of different concentrations of chitosan (the commercial product Kitoflokk-200 (KF), at 2% and 4%), with water (H_2O)-primed and unprimed seeds as control. Foliar application was performed by spraying 100 mL of H_2O

(control), hydrolysed wheat gluten, potato proteins, chitosan and nutrient solution onto plant leaves at weeks three and seven.

Early drought stress (30-58 days after sowing) and late drought stress (60-74 days after sowing) were applied to plants in both biotron and greenhouse trials. Detailed descriptions of agronomic trait measurements during the biotron and greenhouse trials can be found in Papers I and V, respectively.

4.3 Image-based phenotyping

As shown in Figure 3, early root vigour and shoot vigour were phenotyped using cameras at seven days and 20 days after sowing, respectively (Paper I). Images of wheat plants were collected weekly from week four to week 10 after sowing, using a phenocart (Paper V). Data on early root vigour, early shoot vigour and shoot biomass were extracted by the software products RootNav, Easy Leaf Area and ImageJ, respectively. Detailed information on camera set-up and image analyses can be found in Papers I and V.

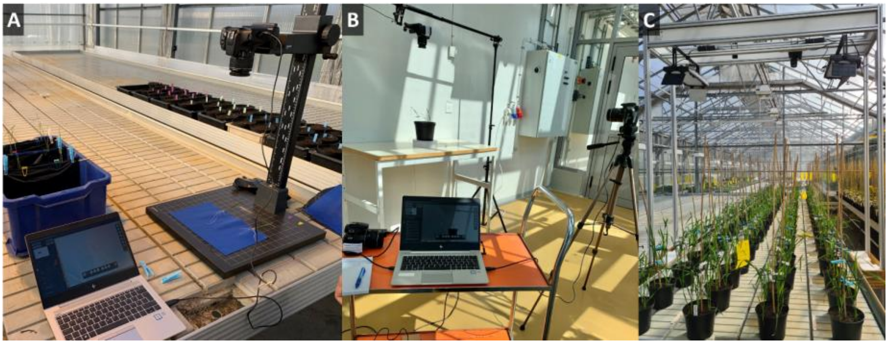

Figure 3. Equipment used in image-based phenotyping of (A) early root vigour and (B) early shoot vigour, and (C) weekly recording of shoot biomass.

4.4 Protein composition analyses

Wheat grain samples were first milled into flour and then subjected to a two-step protein extraction process using sodium dodecyl sulphate (SDS) (Gupta *et al*., 1993). Proteins extracted by the first and second steps were designated SDS-extractable and SDS-unextractable proteins, respectively. Size exclusion-high-performance liquid chromatography (SE-HPLC) was used to analyse these proteins, which were separated into four groups of factions according to their size, *i.e*. large polymeric proteins (LPP), smaller polymeric proteins (SPP), large monomeric proteins (LMP) and smaller monomeric proteins (SMP). These four protein factions were then used to calculate six breadmaking quality-related protein parameters, *i.e.* total SDS-extractable protein (TOTE), total SDS-unextractable protein (TOTU), percentage of SDS-unextractable polymeric protein in total polymeric protein (%UPP), percentage of large SDS-unextractable polymeric protein in total large polymeric protein (%LargeUPP), percentage of large SDS-unextractable monomeric protein in total large monomeric protein (%LUMP), and ratio of total monomeric protein to total polymeric protein (Mon/Pol). Detailed descriptions of protein extraction and equations used to calculate these parameters can be found in Paper II.

4.5 Grain mineral and heavy metal analyses

Flour samples for mineral and heavy metal analyses were first mixed with nitric acid and then digested in an autoclave at 121 °C and 200 kPa for 30 min. After digestion, the samples were diluted 10 times and sent for element content analyses. Concentrations of Zn, Se and Mn were determined by inductively coupled plasma mass spectrometry (ICP-MS), while concentrations of Ca, Cu, Fe, K, Mg, Na, P and S were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Paper III). All heavy metal (Cd, Pb and Hg) concentrations were measured by ICP-MS (Paper IV). Full details of sample preparation and digestion are provided in Papers III and IV.

5. Results and Discussion

5.1 Superior root vigour identified at an early stage

A robust root system at early growth stages is a beneficial attribute that places wheat seedlings in a better position to compete with weeds for limited resources, *e.g.* water and nutrients (Palta *et al*., 2011). Old Swedish wheat cultivars are known for their strong root growth, which is generally assumed to be a positive contributor to their stable performance under drought conditions. However, scientific evidence for this assumption is still lacking. As shown in Figure 4, modern genotypes analysed in this thesis were found to have significantly shorter roots compared with old Swedish cultivars. This reflects one of the typical outcomes of modern breeding programmes, namely greater biomass allocation to above-ground plant parts than roots, as a consequence of yield-oriented selection. This corresponds well with findings in other studies of a decreasing trend in root length from old to modern wheat (Bektas et al., 2016; Bektas and Waines, 2018; Waines and Ehdaie, 2007). The significantly longer roots found in wheat-rye introgression genotypes with 1R and 1RS than 2R genotypes (see Figure 4) suggest the presence of genes contributing to vigorous root growth carried by 1R and 1RS. Boosted rooting ability conferred by introgression of 1RS into wheat genome aligns well with findings in other studies (Liu *et al*., 2020; Sharma *et al*., 2018). This opens up the possibility of utilising 1R and 1RS genotypes as parental lines in breeding programmes to enhance the early environmental resilience of modern wheat.

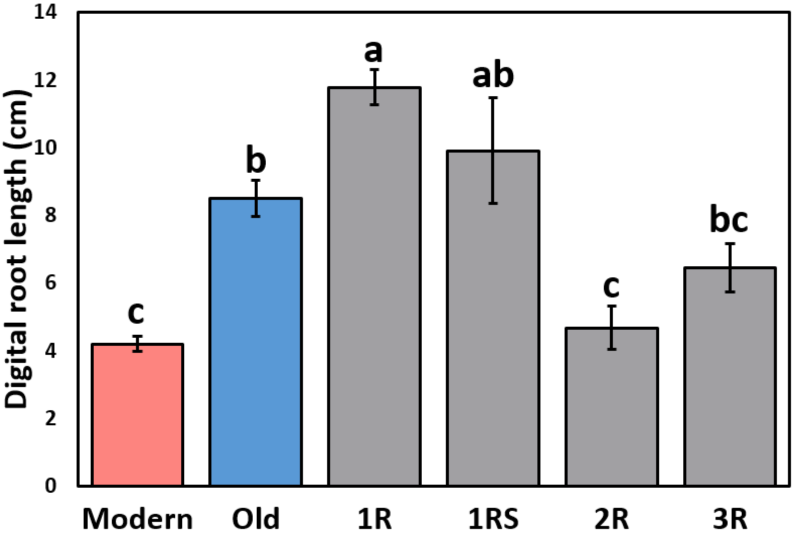

Figure 4. Early root vigour, measured as digital root length, of different genetic sources comprising modern breeding lines, old Swedish cultivars and wheat-rye introgression lines carrying rye chromosomes 1R, 1RS, 2R and 3R. Different lowercase letters on bars indicate statistical significance (p<0.05) in Tukey post-hoc test.

5.2 Effect of early and late drought on agronomic traits, protein quality, mineral composition and heavy metal content

The agronomic traits evaluated in this thesis consisted of two morphological traits (tillers per plant and flag leaf area) and four yield traits (spikes per plant, spike length, grains per plant and grain weight per plant). In general, early drought had a greater impact on the morphological trait flag leaf area, while late drought restricted all yield traits more severely than early drought (Table 1). The different effects of early and late drought observed in this thesis correspond well with previous findings of large adverse impacts of early drought on most plant structural characteristics, while drought imposed after anthesis causes severe yield reductions (Hassan *et al.*, 1987).

Percentage of SDS-unextractable polymeric protein in total polymeric protein (%UPP) was found to be independent of both early and late drought, while TOTE was significantly increased by late drought (Table 1), implying different stress response mechanisms in terms of protein concentration and gluten strength. Protein polymerisation is known to occur during the late grain-filling stage, meaning that gluten strength can be affected by environmental factors such as temperature and nitrogen regime during late grain-filling that alter wheat reproductive stages drastically (Johansson *et al*., 2013; Lama *et al*., 2023). The lack of drought effect on wheat heading date (Paper I) might explain the lack of effect of drought on %UPP observed in Paper II.

Concentrations of Zn and Fe and of the heavy metal cadmium increased under late drought, while no effect was seen on Se concentration (Table 1). Zinc, Fe and Se are all essential for human health (Lim *et al*., 2013; Rayman, 2012), but in plants only Zn and Fe are required for growth and the function of Se is unclear. The non-essential nature of Se for plants might result in a different uptake mechanism compared with Zn and Fe. The elevated Cd concentration under late drought implies a risk of excessive accumulation of this heavy metal under climate change (Table 1).

Table 1. *Agronomic, protein, mineral and heavy metal traits of diverse wheat genotypes grown under different conditions (mean ± standard deviation)*

Trait (units)	Control	Early drought	Late drought
Tillers per plant (n)	3.85 ± 0.65a	3.89 ± 0.98a	4.09 ± 0.79a
Flag leaf area (cm^2)	32.3 ± 7.43a	19.65 ± 4.22c	28.86 ± 9.27b
Spikes per plant (n)	3.42 ± 0.60a	3.14 ± 0.80b	1.62 ± 0.65c
Spike length (cm)	9.10 ± 1.12a	8.46 ± 1.11b	8.03 ± 1.18c
Grains per plant (n)	111.41 ± 25.42a	76.08 ± 21.89b	38.99 ± 21.82c
Grain weight per plant (g)	4.02 ± 0.81a	2.69 ± 0.62b	1.09 ± 0.63c
TOTE ($\times 10^8$)	1.32 ± 0.24b	1.45 ± 0.20b	2.33 ± 0.55a
%UPP (%)	36.04 ± 7.28a	35.93 ± 7.50a	34.97 ± 7.86a
Zn concentration (mg/kg)	15.7 ± 4.59b	20.06 ± 6.04b	37.16 ± 12.18a
Fe concentration (mg/kg)	17.93 ± 4.98b	20.90 ± 5.44b	29.90 ± 8.32a
Se concentration (mg/kg)	0.011 ± 0.0010a	0.0084 ± 0.0065a	0.016 ± 0.015a
Cd concentration (mg/kg)	0.014 ± 0.0055b	0.018 ± 0.0049b	0.030 ± 0.013a

Value within rows followed by different lowercase letters differ significantly (Tukey post-hoc test at $p<0.05$).

5.3 Genotypes with different levels of drought tolerance

In the context of crop cultivation, drought tolerance can be defined as the yield-maintaining ability of a plant. This thesis evaluated the drought tolerance of wheat genotypes using a stress tolerance index (STI) based on grain yield (Fernandez, 1993). In general, there was no significant difference in mean STI values between different genotype groups under early or late drought stress conditions. Compared with early drought, late drought caused a relatively low level of STI in all genotype groups, suggesting a severe impact of late drought on grain yield (Table 2). Surprisingly, among genotypes with the highest 10% of STI values, genotypes carrying chromosome 3R outnumbered other genotype groups, indicating a positive effect of 3R on tolerance to early drought. No clear pattern of a dominant genotype group was found for late drought tolerance. The large root biomass and high number of grains developed in 3R genotypes likely contributed to the observed improvements in final grain weight (Paper I). However, available information on effects of the 3R introgression on drought tolerance is limited. The low transmission rate of 3R (25.0%) during backcrossing might account for this lack of information (Li *et al*., 2018). Only one study has reported potential presence of genes contributing to drought tolerance on chromosome 3R (Mohammadi *et al*., 2003). Thus, increasing the rate of successful transfer of 3R to the wheat genome and wider testing of the tolerance of 3R genotypes could be of great interest in developing climate-resilient varieties.

Table 2. *Average stress tolerance index (STI) of different genotype groups and genotypes with the highest 10% STI values when grown under early drought and late drought conditions (mean ± standard deviation)*

	Early drought		Late drought	
	Genotype (group)	STI value	Genotype (group)	STI value
Mean STI of each genotype group	Modern	0.72 ± 0.10a	Modern	0.38 ± 0.15a
	Old	0.85 ± 0.12a	Old	0.34 ± 0.15a
	1R	0.57 ± 0.15a	1R	0.20 ± 0.10a
	1RS	0.59 ± 0.20a	1RS	0.19 ± 0.13a
	2R	0.66 ± 0.15a	2R	0.39 ± 0.20a
	3R	0.86 ± 0.29a	3R	0.20 ± 0.10a
Genotypes with the highest 10% STI values	257 (3R)	1.29	238 (2R)	0.67
	256 (3R)	1.25	281 (modern)	0.66
	244 (3R)	1.18	204 (old)	0.58
	227 (1RS)	0.98	273 (modern)	0.57
	202 (old)	0.97	201 (old)	0.57
	208 (old)	0.97	270 (2R)	0.51
	254 (3R)	0.97	217 (1R)	0.44

Mean values within columns followed by different lowercase letters differ significantly (Tukey post-hoc test at $p<0.05$).

5.4 Negative relationship between grain yield and protein content

Protein concentration was negatively correlated with grain yield under early and late drought, while no correlation was found under the control condition (Figure 5). Further, the overall negative protein-yield relationship across the three types of growing conditions showed a clear trend for an increase in grain protein concentration with the reduction in grain yield induced by drought. This aligns well with findings in other studies (Cooper *et al*., 2001; Feil, 1997; Simmonds, 1995). In particular, Cooper *et al*. (2001) observed this negative correlation in multi-environmental trials, and found that the genotype × environment (G × E) interaction was a greater contributor to the overall variance in both protein concentration and grain yield than the genotypic effect.

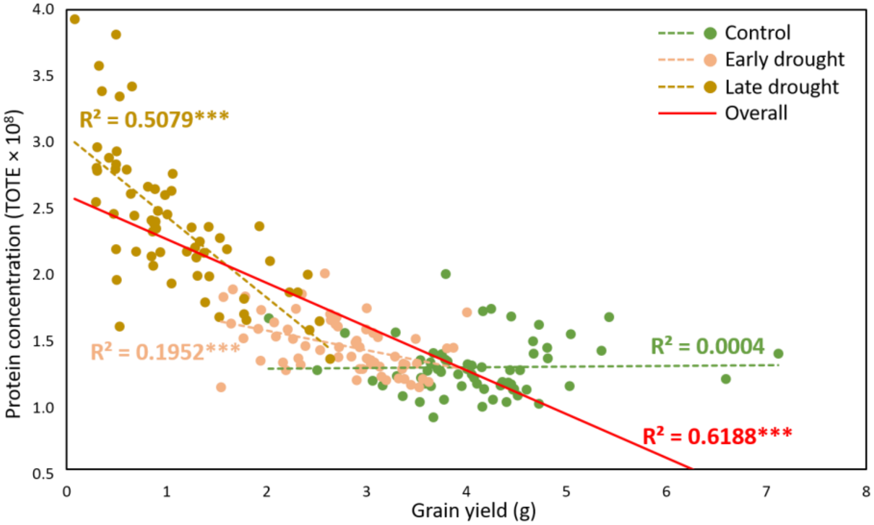

Figure 5. Linear relationship, shown with the coefficient of determination (R^2), between protein concentration and grain yield of 73 wheat genotypes grown under control, early drought and late drought conditions (***: *p*<0.001, **: *p*<0.01, *: *p*<0.05).

5.5 Potential for using alien wheat to improve end-use quality and nutritional value

Modern wheat showed generally lower protein, Zn, Fe and Se concentrations than the other genotype groups analysed (Papers II & III). This reduction in concentrations of the three micronutrients is likely due to long-term selection based on yield performance. Previous studies have also reported lower protein and mineral concentrations in modern wheat than in old landraces, alien species and distant relatives (Hussain *et al*., 2013; Moreira-Ascarrunz *et al*., 2016; Murphy *et al*., 2008). In attempts to identify genetic materials of relevance in improving grain quality, the stability of protein and mineral traits at genotype group level was assessed across different drought conditions based on genotype and genotype-by-environment interaction (GGE).

Genotypes with 3R had the highest TOTE, with similar stability level as 2R and modern genotypes (Figure 6), which corresponds well with results obtained in comparison of TOTE for different genotype groups (Paper II). This demonstrates the potential of rye chromosome 3R for protein content improvement. Further, combined with yield data, genotypes 252 (3R) and 253 (3R) were observed to have both high protein concentration and high grain yield, suggesting possibilities of breeding for improved grain quality without compromising yield. However, all the wheat-rye introgression genotypes were found to have low %UPP compared with old and modern wheat (Figure 6), implying decreased gluten strength induced by introduction of rye chromosomes. Issues with reduced gluten quality, such as weak and sticky dough, have been reported previously for introgression lines carrying 1R and 1RS (Fenn *et al*., 1994; Graybosch *et al*., 1993).

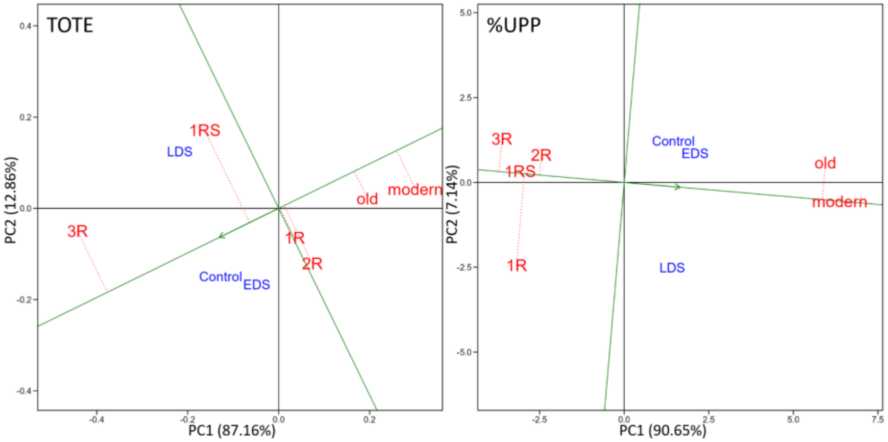

Figure 6. Genotype and genotype-by-environment interaction (GGE) biplots for protein composition traits, total SDS-extractable proteins (TOTE) and percentage of SDS-unextractable polymeric proteins in total polymeric proteins (%UPP) in wheat grain grown in control, early drought stress (EDS) and late drought stress (LDS) conditions.

Genotypes with 3R generally displayed high and stable grain Zn and Fe concentrations across the three sets of growing conditions, while 1R genotypes were specifically high in Se concentration (Figure 7). Notably, genotypes 250 (3R) and 251 (3R) were found to have considerably high and stable concentrations of both Zn and Fe, while several 1R and 1RS genotypes (215, 220, 221, 222 and 229) had particularly high and stable Se concentration (Paper III). Zinc and iron have strong health-related effects, with deficiency of these micronutrients affecting more than two billion people around the world (Bouis and Saltzman, 2017; Vasconcelos et al., 2017), while approximately one billion people are living under Se deficiency (Combs, 2001). With continued rapid urbanisation, increasing food demand and an unstable future climate, nutrient deficiency is expected to increase, especially in developing countries (Senker, 2011), resulting in lack of micronutrients and impaired physical and mental human health (Tulchinsky, 2010). Thus, biofortification to improve the nutritional value of modern wheat is urgently needed, and exploiting the micronutrient-accumulating wheat-alien lines identified in this thesis within future breeding could be a solution in the context of climate change.

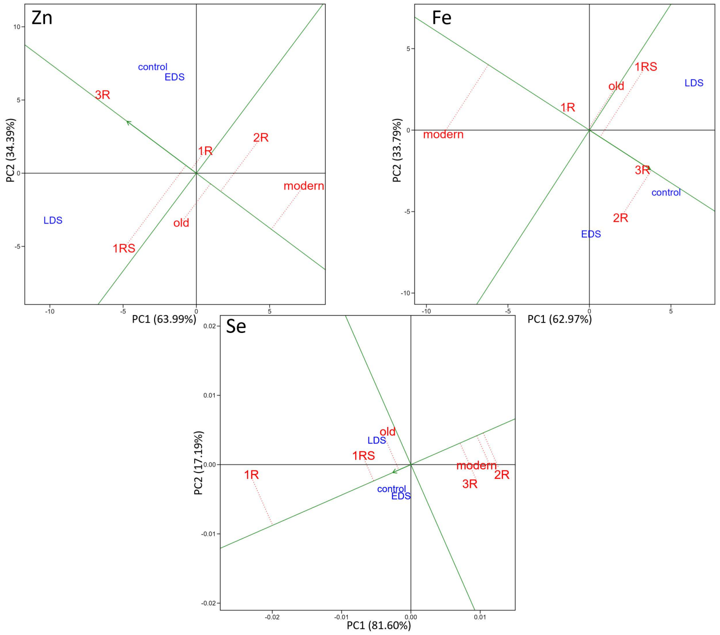

Figure 7. Genotype and genotype-by-environment interaction (GGE) biplots for zinc (Zn), iron (Fe) and selenium (Se) concentrations in wheat grain grown in control, early drought stress (EDS) and late drought stress (LDS) conditions.

5.6 Risk of excessive heavy metal accumulation in 1R and old genotypes

An increasing trend in Cd concentration was generally observed in grains of modern, 1R, 1RS and 2R wheat genotypes under late drought (Figure 8A), indicating an increasing risk of elevated Cd content in wheat products under climate change. Narrowed down to specific genetic backgrounds, this thesis identified an alarming trend for the 1R introgression to be accompanied by elevated Cd accumulation in grain (Figure 8A). Genotypes with 1R showed significantly higher grain Cd concentrations than other genotype groups under control conditions, while they showed significantly higher concentrations than other wheat-rye introgression lines under early and late drought (Figure 8A). There was thus a consistently high level of Cd in grains of 1R genotypes, regardless of the growing environment. Grain Cd levels were lower in 1RS genotypes than in 1R genotypes, suggesting that the genes contributing to elevated Cd uptake may be contained in the long arm of 1R. Transferring 1R into the wheat genome has been reported to confer advantageous characteristics in several regards, *e.g.* vigorous early root (Paper I), increased final root biomass (Sharma *et al.*, 2018), improved grain yield (Ren *et al.*, 2018) and enhanced disease resistance (Mago *et al.*, 2002), but few studies have examined the relationship between 1R introgression and heavy metal accumulation. In this thesis, old genotypes were found to have significantly higher grain Cd concentrations than modern, 1RS, 2R and 3R genotypes when grown under control conditions (Figure 8A), suggesting a similar risk of excessive Cd accumulation. Interestingly, both 1R and old genotypes displayed superior rooting ability compared with the other genotype groups during early growth stages (Paper I). Thus, the high levels of Cd observed in grains of 1R and old genotypes are likely explained by their vigorous root growth, as confirmed by the significantly positive linear relationship between root vigour (Paper I) and Cd concentration (Paper IV) (Figure 8B). In crop breeding and cultivation, a large root system is usually considered to be a beneficial characteristic, especially in terms of environmental adaptation, and wheat alien and ancient genetic materials with a larger root system have been used to achieve improvements in climate resilience and disease resistance (Crespo-Herrera *et al.*, 2017; Liu *et al.*, 2020). To minimise the risk of food toxicity, the results presented in this thesis indicate a need for rigorous assessments of heavy metal content in wheat varieties carrying chromosome 1R or bred from old germplasm.

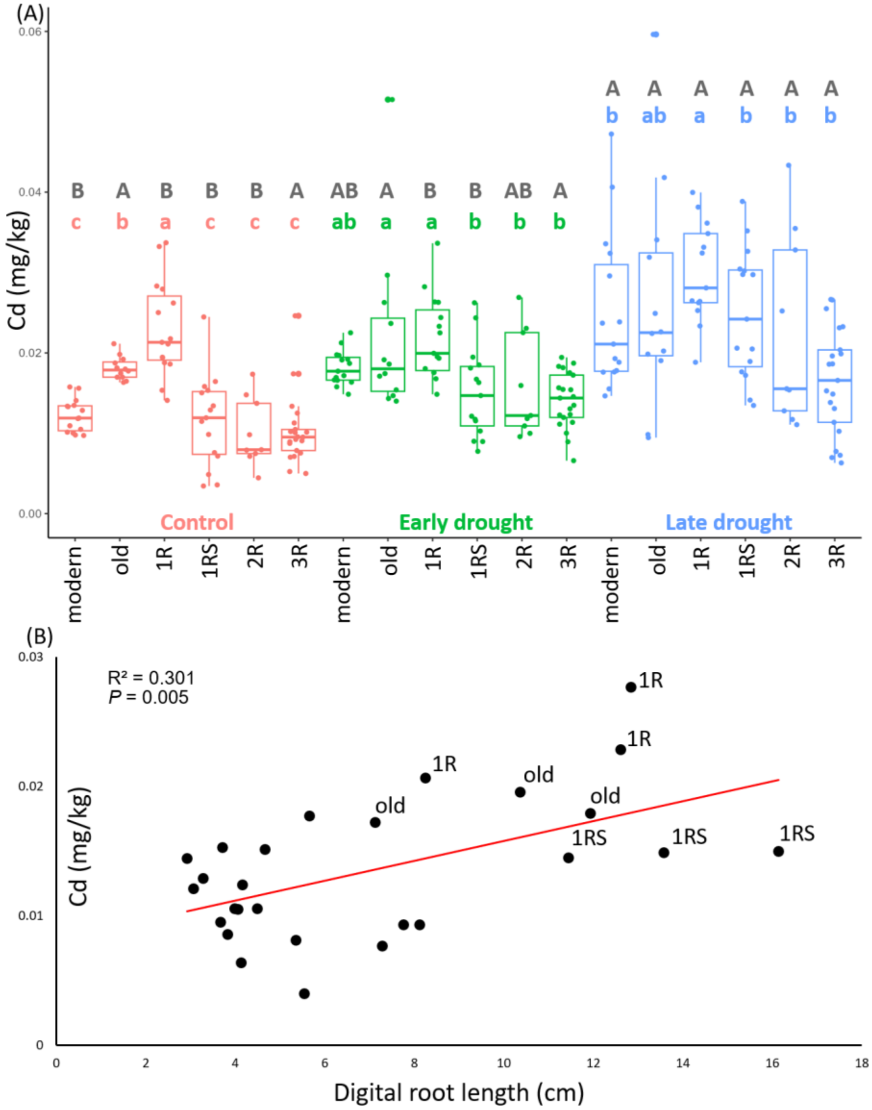

Figure 8. (A) Cadmium (Cd) concentration in grains of different wheat genotype groups grown in control, early drought and late drought conditions. Lowercase letters indicate the results of mean comparison between genotype groups within each treatment, while capital letters indicate the results of mean comparison of the same genotype group between different treatments (LSD post-hoc test at $p<0.05$). (B) Linear regression (R^2 = coefficient of determination) of root vigour (digital root length) and grain Cd concentration in plants grown under control conditions.

5.7 Is biostimulant application a solution for drought tolerance?

No significant effect of seed-primed biostimulants was found in terms of grains per plant (GPP) or grain weight per plant (GWPP) under control conditions (Figure 9). However, a significant increase in GWPP in the modern genotype 276 was obtained by KF4% priming under early drought, indicating chitosan-induced tolerance to early drought.

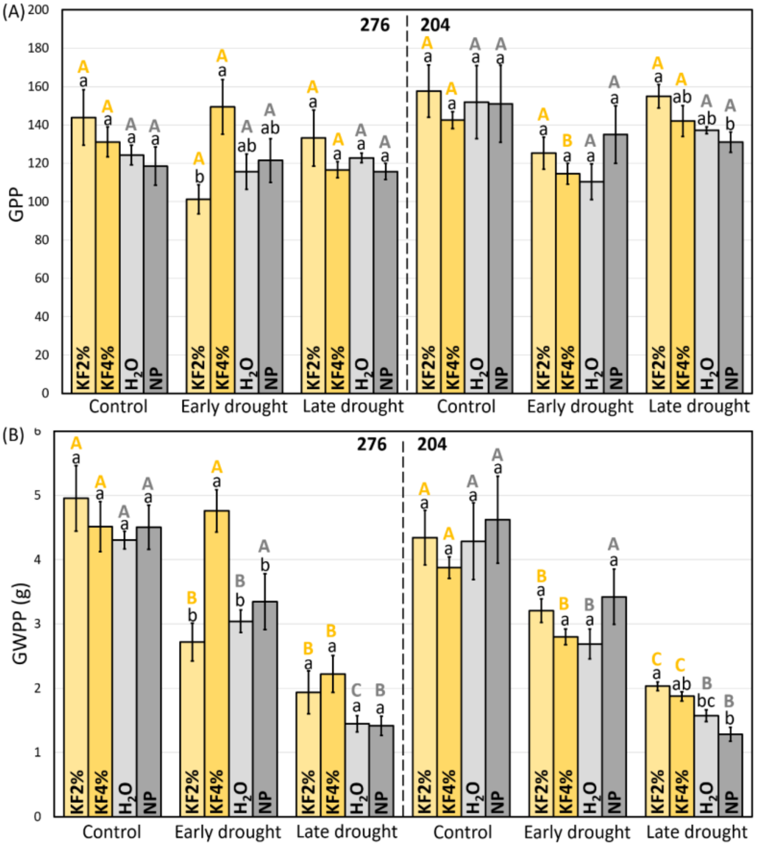

Figure 9. (A) Grains per plant (GPP) and (B) grain weight per plant (GWPP) of wheat genotypes 276 and 204 primed with biostimulants under control, early drought and late drought. KF: Kitoflokk-200 at 2% or 4%, H_2O: water priming, NP: no priming. Means for individual biostimulant treatments in the different drought treatments with different capital letters differ significantly. Means of different biostimulant treatments within each drought treatment with different lowercase letters differ significantly (LSD post-hoc test at $p<0.05$).

The old genotype 204 showed enhanced tolerance to late drought, with increased GPP and GWPP under KF2% priming compared with non-primed plants (Figure 9). These results align well with previous reports of significantly increased grain number in wheat plants grown from seed dressed with chitosan oligosaccharides (Wang *et al*., 2015).

Foliar application of potato proteins (PP) significantly increased GWPP in the modern genotype 276 compared with the H_2O leaf spray under control conditions (Figure 10). Plants sprayed with hydrolysed wheat gluten (HWG) showed higher GWPP than plants sprayed with H_2O under late drought, suggesting improved drought tolerance triggered by HWG. On the other hand, the old genotype 204 exhibited enhanced GPP following foliar application of PP under late drought. Potato proteins and hydrolyzed wheat glutens have not been widely applied in wheat cultivation to date, and relevant reports are therefore lacking. However, both are the by-product of starch extraction in the food industry, giving them advantages such as easy accessibility and relatively low cost (Asrarkulova and Bulushova, 2018; Capezza et al., 2019). The positive effects on wheat yield traits observed under drought stress indicate good potential for the use of these biostimulants to cope with the yield pressure brought about by climate change.

Overall, the biostimulants tested in this thesis, *i.e*. chitosan, hydrolyzed wheat glutens and potato proteins, were all found to have positive effects on yield traits under drought conditions (Figure 10). Making use of these biostimulants could help to address food security issues and also environmental issues, as they are generally treated as wastes during industrial processing. However, more research is needed to identify the optimal concentration and most effective application method for different types of biostimulants.

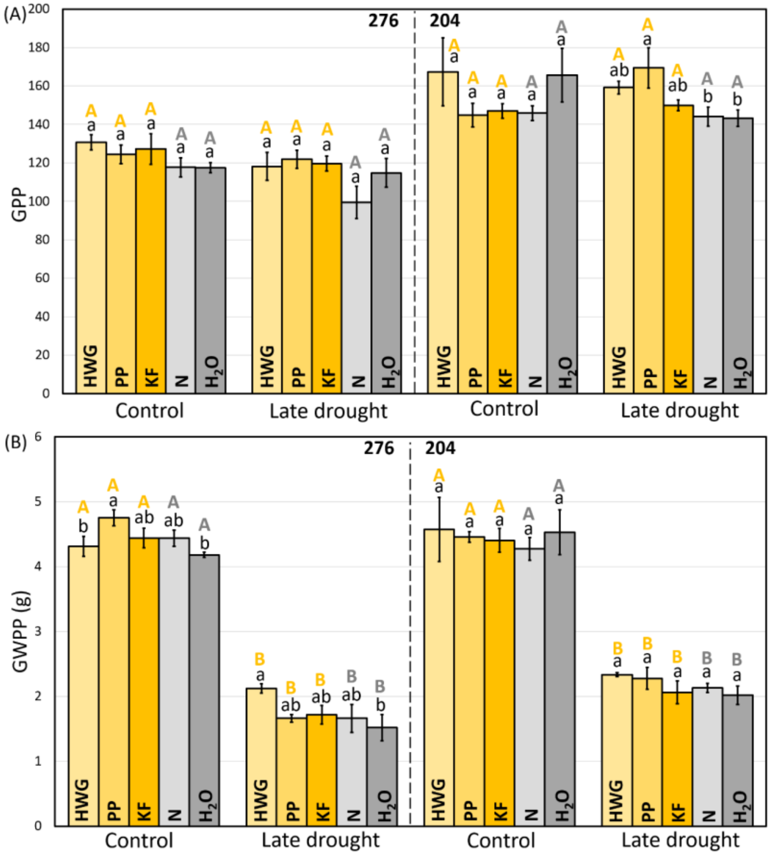

Figure 10. (A) Grains per plant (GPP) and (B) grain weight per plant (GWPP) of wheat genotypes 276 and 204 following foliar application of biostimulants under control and late drought. HWG: hydrolysed wheat gluten, PP: potato proteins, KF: 2% Kitoflokk-200, N: nutrient solution, H_2O: water. Means for individual biostimulant treatments in the different drought treatments with different capital letters differ significantly. Means of different biostimulant treatments within each drought treatment with different lowercase letters differ significantly (LSD post-hoc test at $p<0.05$).

6. Conclusions

In this thesis, the effects of drought stress at early and late stages of wheat growth on various aspects relevant to food security, *i.e.* grain yield, breadmaking quality, nutritional value and toxicity, were comprehensively examined in groups of wheat genotypes sourced from different genetic backgrounds. The possibility of utilising biostimulants to alleviate drought impacts on wheat yield performance was also evaluated. The main conclusions were as follows:

- Late drought had a greater impact on wheat yield and grain quality traits, with yield traits decreasing and protein and mineral concentrations increasing, whereas early drought mainly restricted morphological traits.

- Old and 1R genotypes showed superior root vigour at seedling stage, while genotypes with chromosome 3R from rye showed high tolerance to early drought.

- There was generally a negative correlation between protein concentration and grain yield, but two 3R genotypes (252 and 253) showed high protein concentration and high grain yield under control, as well as high stability of protein concentration across drought conditions.

- Genotypes with 3R had the highest grain Zn concentrations and generally high Fe concentrations, with 250 (3R) and 251 (3R) showing high and stable concentrations of both Zn and Fe, while 1R genotypes had high grain Se concentrations.

- Late drought generally increased Cd concentration in grains. The high levels of Cd in 1R and old genotypes indicated a high risk of excessive grain Cd accumulation that may be linked to a larger root system in 1R and old genotypes.

- Seed priming with chitosan (KF4%) had a positive effect on grain weight per plant in genotype 276 (modern) under early drought, while seed priming with KF2% had a positive effect on number of grains per plant and grain weight per plant in genotype 204 (old) under late drought. Compared with H_2O spraying, foliar application of hydrolysed wheat glutens increased grain weight per plant of genotype 276 under control conditions, while foliar application of potato proteins increased number of grains per plant in genotype 204 under late drought.

7. Future perspectives

As a complement to the findings presented in this thesis, future studies should conduct analyses at molecular level. Acquisition of genotypic data for the lines used in this thesis would enable an association study for phenotypic data obtained under well-watered and drought conditions. Through this, genes relevant to root vigour, drought tolerance, breadmaking quality and nutritional value that are potentially present on rye chromosomes 1R, 1RS and 3R could be located and specifically identified. The 3R genotypes identified as possessing high yield, high protein concentration and high mineral concentration are particularly worth further exploration. However, the risk of excessive heavy metal uptake by old and 1R wheat under climate change needs to be addressed. Instead of incorporating the entire 1R chromosome, using 1RS might be a solution, due to the lower Cd content observed in 1RS genotypes in this thesis, and most of the advantageous genes are found in the short arm of 1R. Further possibilities could be explored by transferring 1R in combination with other rye chromosomes such as 2R and 3R, which demonstrated promise in lowering heavy metal accumulation. Beyond those long-term solutions, using biostimulants to sustain plant growth under abiotic stress can serve as a fast and cost-effective solution. Future studies on this topic should test the effectiveness of different biostimulants and seek to identify the most suitable concentration and application mode.

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Popular science summary

Climate change is now affecting agricultural production around the world, mainly through global warming causing an increasing number of adverse weather events such as heatwaves and droughts. Wheat is the most cultivated crop in Sweden, (with over three million tonnes of grain typically produced annually. A safe and stable supply of wheat therefore plays a crucial role in Swedish food security. However, wheat productivity is now being destabilised by changes in weather patterns during the Swedish growing season. A good example is the hot, dry summer of 2018, which led to a 51% reduction in total Swedish wheat production in that year.

In this thesis, wheat lines from different genetic backgrounds were placed under drought conditions and their performance was analysed in terms of aspects relevant to food security, *i.e.* grain yield, breadmaking quality, nutritional value and toxicity. The results showed that drought stress occurring during late stages of wheat growth, corresponding to the typical time of Swedish summer drought, severely decreased grain yield. Interestingly, the different genetic materials tested showed contrasting behaviours in terms of their responses to drought. Old Swedish cultivars and wheat lines carrying the rye chromosome 1R displayed superior root vigour, improving their resilience to drought. However, this better rooting ability was also associated with elevated uptake of cadmium, a heavy metal, raising issues of food toxicity. Modern wheat exhibited high grain yield but the grain had low protein and mineral content. Wheat-rye introgression lines with chromosome 1R had prominently higher concentrations of the macronutrient selenium than other lines. An exciting finding was that lines carrying rye chromosome 3R showed several promising characteristics, including tolerance to early drought, high yield, high grain protein concentration and high zinc and iron concentrations in grain.

In an alternative approach to improve resilience to drought, different biostimulants (chitosan, hydrolysed wheat gluten and potato proteins) were applied to the wheat crop by seed priming and foliar application. The results showed improved grain yield of treated wheat plants grown under drought conditions, suggesting the possibility of combating climate change using bioactive compounds.

The novel information provided in this thesis on the effects of drought on different parameters in wheat can be useful in commercial applications. In genetic approaches, the wheat lines identified as having high and stable performance in terms of beneficial crop parameters can be used as genetic materials to widen the tolerance spectrum of modern wheat. In agronomic approaches, the resilience of wheat to varying environments can be enhanced by external application of suitable biostimulants.

Populärvetenskaplig sammanfattning

Klimatförändringarna påverkar nu jordbruksproduktionen runt om i världen, främst genom att den globala uppvärmningen orsakar ett ökande antal ogynnsamma väderhändelser som värmeböljor och torka. Vete är den mest odlade grödan i Sverige (med över tre miljoner ton spannmål som produceras årligen). En säker och stabil tillgång på vete spelar därför en avgörande roll för den svenska livsmedelsförsörjningen. Men vetets produktivitet destabiliseras nu av förändringar i vädermönstren under den svenska växtsäsongen. Ett bra exempel är den varma och torra sommaren 2018, som ledde till en 51-procentig minskning av den totala svenska veteproduktionen det året.

I denna avhandling placerades vetelinjer från olika genetiska bakgrunder under torra förhållanden och deras prestanda analyserades med avseende på aspekter som är relevanta för livsmedelsförsörjningen, dvs. spannmålsskörd, brödkvalitet, näringsvärde och toxicitet. Resultaten visade att torkstress som uppstod under vetets sena tillväxtstadium, vilket motsvarar den typiska tidpunkten för svensk sommartorka, kraftigt minskade spannmålsskörden. Intressant nog uppvisade de olika genetiska material som testades kontrasterande beteenden när det gäller deras svar på torka. Gamla svenska sorter och vetelinjer som bär på rågkromosomen 1R uppvisade överlägsen rotkraft, vilket förbättrade deras motståndskraft mot torka. Denna bättre rotförmåga var emellertid också förknippad med ett förhöjt upptag av kadmium, en tungmetall, vilket väcker frågor om livsmedelstoxicitet. Modernt vete gav hög skörd, men spannmålen hade lågt protein- och mineralinnehåll. Introgressionslinjer av vete och råg med kromosom 1R hade påtagligt högre koncentrationer av makronäringsämnet selen än andra linjer. Ett spännande resultat var att linjer med rågkromosom 3R uppvisade flera lovande egenskaper, bland annat tolerans mot tidig torka, hög skörd, hög proteinhalt i kornet och höga zink- och järnkoncentrationer i kornet.

I ett alternativt tillvägagångssätt för att förbättra motståndskraften mot torka applicerades olika biostimulanter (kitosan, hydrolyserat vetegluten och potatisproteiner) på vetegrödan genom utsäde och bladapplicering. Resultaten visade en förbättrad spannmålsskörd för behandlade veteplantor som odlats under torra förhållanden, vilket tyder på möjligheten att bekämpa klimatförändringar med hjälp av bioaktiva föreningar.

Den nya information som ges i denna avhandling om effekterna av torka på olika parametrar i vete kan vara användbar i kommersiella tillämpningar. I genetiska metoder kan de vetelinjer som har identifierats med hög och stabil prestanda när det gäller fördelaktiga grödparametrar användas som genetiska material för att bredda toleransspektrumet för modernt vete. Med agronomiska metoder kan vetets motståndskraft mot varierande miljöer förbättras genom extern applicering av lämpliga biostimulanter.

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Article

Climate Change Impact on Wheat Performance—Effects on Vigour, Plant Traits and Yield from Early and Late Drought Stress in Diverse Lines

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Abstract: Global climate change is threatening wheat productivity; improved yield under drought conditions is urgent. Here, diverse spring-wheat lines (modern, old and wheat-rye introgressions) were examined in an image-based early-vigour assay and a controlled-conditions (Biotron) trial that evaluated 13 traits until maturity. Early root vigour was significantly higher in the old Swedish lines (root length 8.50 cm) and introgressed lines with 1R (11.78 cm) and 1RS (9.91 cm) than in the modern (4.20 cm) and 2R (4.67 cm) lines. No significant correlation was noted between early root and shoot vigour. A higher yield was obtained under early drought stress in the 3R genotypes than in the other genotype groups, while no clear patterns were noted under late drought. Evaluating the top 10% of genotypes in terms of the stress-tolerance index for yield showed that root biomass, grains and spikes per plant were accountable for tolerance to early drought, while 1000-grain weight and flag-leaf area were accountable for tolerance to late drought. Early root vigour was determined as an important focus trait of wheat breeding for tolerance to climate-change-induced drought. The responsible genes for the trait should be searched for in these diverse lines. Additional drought-tolerance traits determined here need further elaboration to identify the responsible genes.

Keywords: spring wheat; early vigour; image-based phenotyping; drought; stress-tolerance index; yield performance

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1. Introduction

As one of the major staple food sources around the world, wheat provides approximately 20% of the calories and proteins to the daily human diet [1]. According to the latest update in 2020 by the Food and Agriculture Organization of the United Nations (FAO), 219 million ha were harvested, which makes wheat the most widely grown crop; meanwhile, the global production of 761 million tonnes strengthens its position as the world's second-largest crop (https://www.fao.org/faostat/en/#data/QCL, accessed on 26 February 2022). With the pronounced global climate changes (i.e., rising temperature), prolonged shortages of water supply (drought stress) are becoming increasingly frequent, thereby depleting the ecophysiological performance of plants [2,3]. Therefore, drought has been placed at the top of the environmental stresses due to its severe impact on crop productivity as compared to other natural abiotic stresses [4]. Severe drought-induced yield loss in wheat has been reported in several regions of the world [5–7]. Due to the unpredictability of the natural environment, drought stress can threaten a wheat plant at any growth stage throughout its entire life cycle. Late drought has been reported to have a more significant impact on yield loss than early drought [8]. However, in some countries such as Sweden and Denmark, spring wheat is regularly affected by drought during late spring in the early stages of crop development, when the roots have not been fully developed [9]. Therefore, to better understand the mechanism of drought tolerance, the impact of early stress on some phenological and yield-related morphological traits should not be ignored.

Water deficit inhibits the growth of plants by inducing changes of different types, i.e., to the physiological, biochemical, morphological, and molecular characteristics [10]. Many traits besides yield are significantly influenced by drought stress, i.e., flag-leaf area [11], root and plant biomass [12,13], days to anthesis and tillers per plant [14]. To screen for genotypes with high yield potential under stress conditions, a stress-tolerance index (STI) was developed and is used as an effective selection criterion [15]. With the predicted climate change, the need to improve the drought tolerance of wheat has become necessary in many regions of the world.

In addition to traditional labour-intensive trait measurements, scientists have introduced novel sensor-based non-invasive phenotyping techniques in order to investigate plants more efficiently. Various high-throughput phenotyping systems have been established for the study of agronomy traits, i.e., proximal-sensing carts [16], field-scanner systems [17], unmanned aerial systems [18], and automated, standalone systems for controlled growth conditions [19]. Using such systems, the early growth of wheat was found to be correlated to the tolerance to drought conditions, as the tolerant lines tended to display fast early growth [20]. Moderate correlations were identified between early root traits from controlled climatic conditions and drought scores from field trials [13].

The commonly used term 'wheat' usually refers to bread wheat (*Triticum aestivum* L.) that belongs to the tribe Triticeae and the family Poaceae [21,22]. Over years of domestication and breeding, several types of wheat have been developed for different purposes. Modern cultivars mainly aim for a high and stable yield while old breeding lines, landraces and primitive forms of wheat might contain genes of relevance to sustain the varying climate changes [23,24]. Furthermore, wild relatives and landraces of wheat are being used as a unique source of genetic variation to compensate for the low diversity of modern cultivars [25,26]. The successful transfer of genes from the non-*Triticum* species of rye (*Secale cereale*) have yielded disease-resistant wheat cultivars [27–29]. In addition to the disease resistance, the rye chromosome 1RS that was transferred to wheat was reported to carry genes that relate to root biomass [30,31], which could potentially improve tolerance to drought stress.

The present study aimed to use the performance of a broad set of phenotypic traits to characterize drought-stress tolerance in a wide variety of modern, ancient, old, and alien introgressed spring-wheat lines. Furthermore, drought-responding characteristics were related to the genetic background of the material. For successful evaluation, plants were subjected to early or late drought stress in controlled conditions and the performance of the plants was evaluated at the seedling and maturity stage using a combination of classical agronomic traits, including the calculation of STI, and an image-based phenotyping technique. The hypothesis behind this study was that the genes are present in a sufficiently genetically broad wheat material, so that drought-stress tolerance and the genetic background for such tolerance can be identified.

2. Results

2.1. Early Root and Shoot Development

ANOVA clearly showed that both digital-root length (DRL) and digital-leaf area (DLA) varied significantly ($p < 0.001$) among the evaluated genotypes (Table S1), with values ranging from 2.83 cm to 16.13 cm for DRL and from 4.03 cm^2 to 12.52 cm^2 for DLA. No significant correlation was found between the DRL and DLA values, indicating a probability that early root and shoot traits are regulated by separate genetic mechanisms.

ANOVA followed by mean comparisons with the Tukey post-hoc test to compare DRL and DLA in the wheat genotypes of different genetic backgrounds (Figure 1) further verified the separate genetic mechanisms behind early root and shoot growth. Clear significant differences were noted for DRL among the genotype groups, with the significantly longest roots in the old Swedish breeding lines (8.50 cm), the wheat-rye introgression lines with chromosome 1R (11.78 cm), and 1RS (9.91 cm), as compared to the modern cultivars (4.20 cm) and genotypes with chromosome 2R (4.67 cm; Figure 1a). Differently, the significantly highest DLA was noted for the old lines (9.08 cm^2) as compared to the genotypes with chromosome 1RS (6.37 cm^2; Figure 1b) but no significant differences in DLA were found between the modern and old lines or among the introgression lines. Thus, the genes for early root vigour seemed to be present in the old Swedish breeding lines and on chromosome 1R and 1RS, while no presence of similar early vigour genes for shoot growth was indicated.

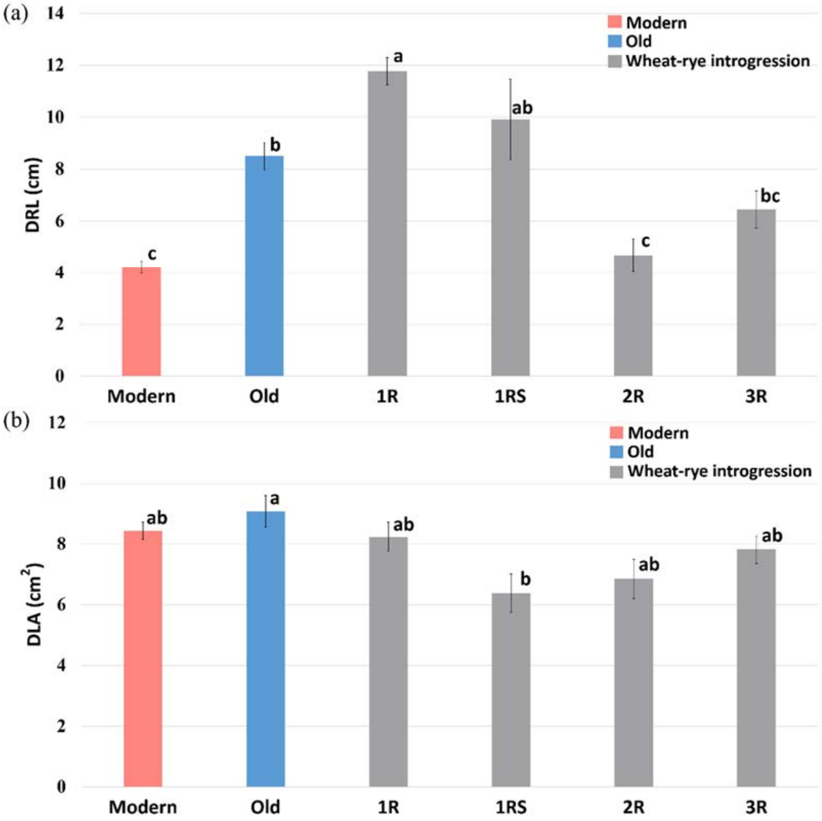


Figure 1. Comparisons of early crop vigour measured as (**a**) digital-root length (DRL) and (**b**) digital-leaf area (DLA) in spring-wheat lines of different genetic background, including modern lines, old Swedish lines and wheat-rye introgression lines with different chromosomes from rye. The results of Tukey post-hoc test are presented by applying a compact letter display at $p < 0.05$. Modern = approved cultivars and breeding lines received from company Lantmännen, Old = cultivars released from 1928 to 1990, 1R = introgressions of chromosome 1R, 1RS = introgressions of chromosome 1RS, 2R = introgressions of chromosome 2R, 3R = introgressions of chromosome 3R.

The top 10% of genotypes (229, 216, 224, 227, 230, 219 and 221) for DRL all contained 1RS and showed values ranging between 12.84 cm and 16.13 cm (Figure 2a) while the top 10% of genotypes (197, 198, 200, 201, 222, 257 and 267) for DLA were spread among the genotype groups, i.e., old Swedish breeding lines and wheat-rye introgression lines with 1R, 2R and 3R, and the values ranged between 10.95 cm^2 and 12.52 cm^2 (Figure 2b).

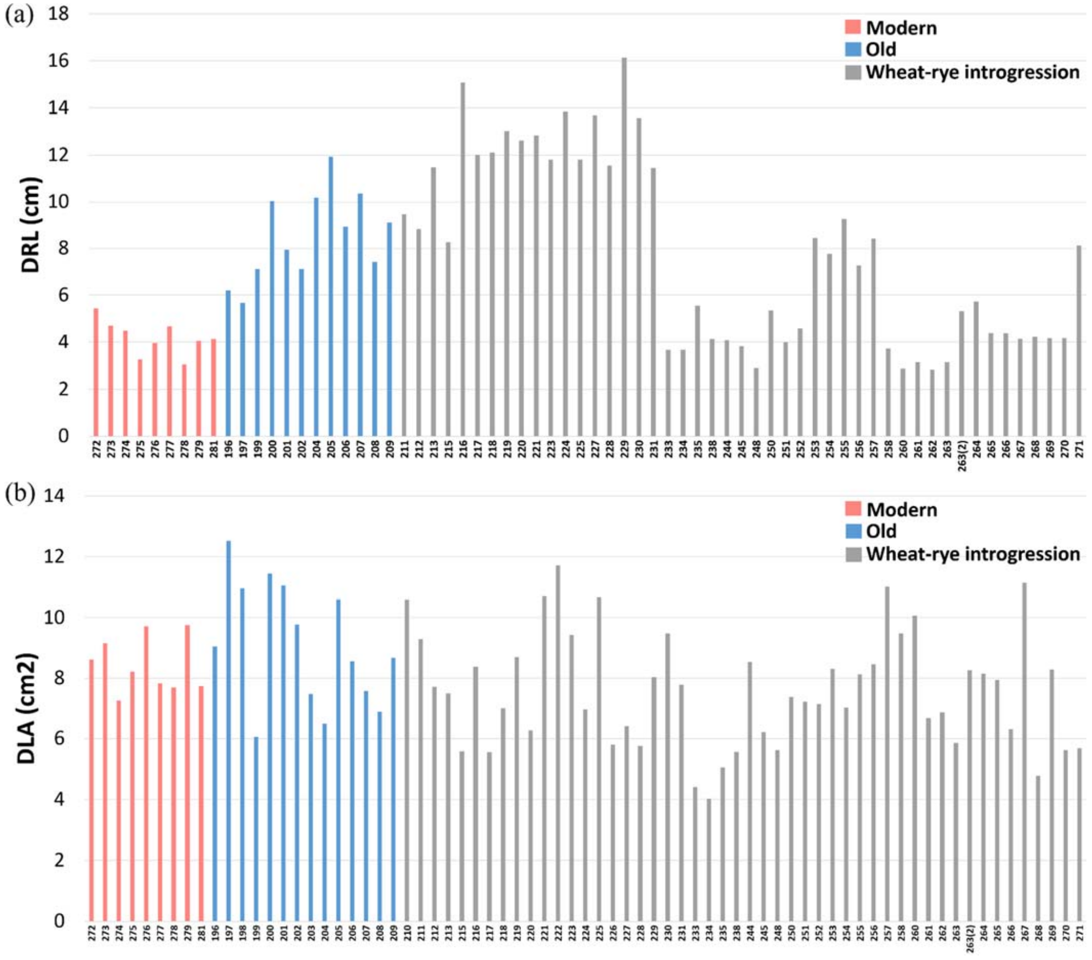


Figure 2. Comparisons of early crop vigour measured as (**a**) digital-root length (DRL) and (**b**) digital-leaf area (DLA) among genotypes including modern lines (approved cultivars and breeding lines received from company Lantmännen), old Swedish lines (cultivars released from 1928 to 1990) and wheat-alien introgression lines (with 1R, 1RS, 2R and 3R). Due to the unstable germination power, five genotypes (198, 203, 210, 222, 226) were missing from the DLA data.

2.2. Relationships between Drought Stresses and Plant Traits

The ANOVA verified a significant effect of the drought treatments on all studied traits, i.e., root biomass (RB), days to heading (DTH), days to anthesis (DTA), tillers per plant (TPP), spikes per plant (SPP), productive spikes per plant (PSPP), flag-leaf area (FLA), spike length (SPL), 1,000-grain weight (TGW), grains per spike (GPS), grains per plant (GPP), grain weight per spike (GWPS), and grain weight per plant (GWPP) (Table S2). A comparison of the mean values of the traits after drought stress at different growth stages (early and late) indicated a significant change in most of them due to the drought treatment (Table S3).

From the principal-component analyses (PCAs), with PC1 representing 39.5% and PC2 19.7% of the variation, the effects of the treatments were well differentiated along with the first principal component (PC1). Basically, all the measured traits were significantly and positively related with the control group (C) of plants (negative PC1), with a decrease in most traits with early drought stress (EDS) and an even larger decrease for late drought stress (LDS). PC2 generally differentiated the variation among the genotypes within a treatment for the evaluated traits (Figure 3).

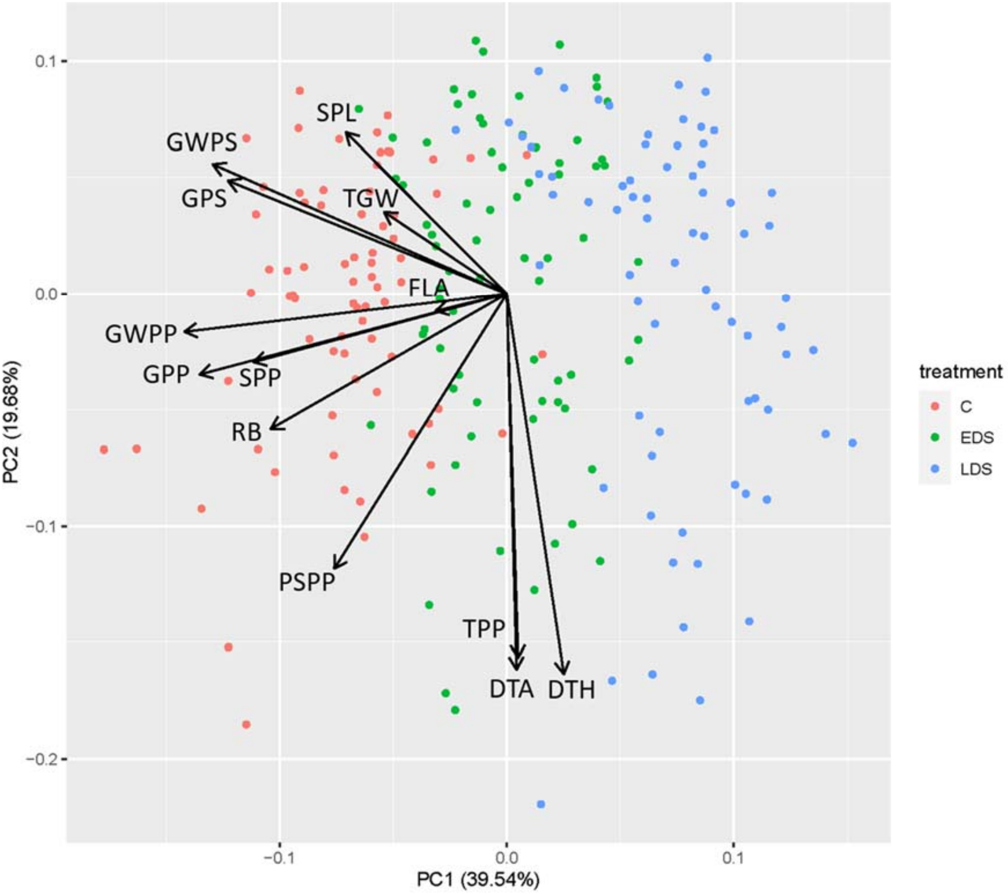
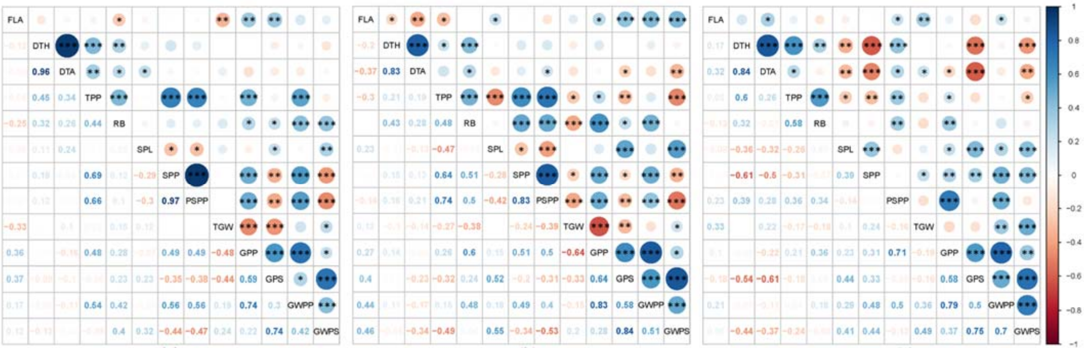


Figure 3. Biplot from principal-component analysis (PCA) for traits, root biomass (RB), days to heading (DTH), days to anthesis (DTA), tillers per plant (TPP), spikes per plant (SPP), productive spikes per plant (PSPP), flag-leaf area (FLA), spike length (SPL), 1000-grain weight (TGW), grains per spike (GPS), grains per plant (GPP), grain weight per spike (GWPS), and grain weight per plant (GWPP) in 73 spring-wheat genotypes under control (C), early drought stress (EDS) and late drought stress (LDS).

2.3. Relationships among Investigated Traits

The present study showed several significant correlations among the non-yield traits (FLA, DTH, DTA, TPP and RB) and yield traits (SPL, SPP, PSPP, TGW, GPP, GPS, GWPP and GWPS; Figure 4). In the non-stressed plants (C), the non-yield traits correlated mostly significantly and positively with GWPP and GPP, while negative correlations were mainly lacking between the non-yield and yield traits (Figure 4a).



(a) (b) (c)

Figure 4. Correlation among morphological, phenological and yield traits flag-leaf area (FLA), days to heading (DTH), days to anthesis (DTA), tillers per plant (TPP), root biomass (RB), spike length (SPL), spikes per plant (SPP), productive spikes per plant (PSPP), 1,000-grain weight (TGW), grains per plant (GPP), grains per spike (GPS), grain weight per plant (GWPP), grain weight per spike (GWPS) studied in wheat under (**a**) control (C), (**b**) early drought stress (EDS) and (**c**) late drought stress (LDS). ***: sig. < 0.001, **: sig. < 0.01, *: sig. < 0.05.

For the EDS plants, a higher number of significant positive correlations were found between non-yield (especially FLA and RB) and yield traits as compared to C plants (Figure 4b).

For the LDS plants, a higher number of significant negative correlations were found for DTH and DTA with the yield traits when compared to the C plants. In comparison, a lower number of significant positive correlations were found for TPP and RB with the yield traits (Figure 4c).

2.4. Genotypic Differences in Reactions to Drought Stresses

A large variation was obtained in plant performance among the genotypes after drought stress. As can be seen from Figure 5, the yield (GWPP) was found to mainly decrease due to EDS but even more so due to LDS, although a large and significant variation was obtained among the genotypes. The ranges of yield under C, EDS and LDS were from 2.02 g to 7.12 g, 1.54 g to 4.00 g and 0.08 g to 2.63 g, respectively which further verified the effects of the drought treatments presented by the mean comparison and PCA (Table S3 and Figure 3).

ANOVA followed by mean comparisons with the Tukey post-hoc test to compare the STI values of DTH, DTA, FLA, TPP, RB, SPP, PSPP, SPL, TGW, GPS, GWPS, GPP and GWPP revealed significant variations among the different genotype groups (modern, old, 1R, 1RS, 2R and 3R) under the two drought treatments (Table S4).

Under EDS treatment, basically, the modern genotypes significantly showed the highest STIs for GPS (0.95) and GWPS (0.96), while the genotypes with chromosome 1RS showed the generally lowest STIs of the yield traits (SPL: 0.80, GPS: 0.47 and GWPS: 0.54). Furthermore, a significantly contrasting performance in RB was noted between the genotypes with chromosome 3R (0.63) and the modern genotypes (0.14).

Under LDS treatment, higher STIs of yield traits were noted in the modern genotypes (SPP: 0.69, GPS: 0.75, GWPS: 0.54 and GWPP: 0.38) as compared to the other genotype groups, while the lowest yield performances were found in the genotypes with chromosome 1RS (SPL: 0.75, GPS: 0.35 and GPP: 0.21) and 3R (SPP: 0.36, TGW: 0.61 and GWPS: 0.24). A significantly contrasting performance in RB was noted between the genotypes with chromosome 3R (0.66) and the old genotypes (0.18).

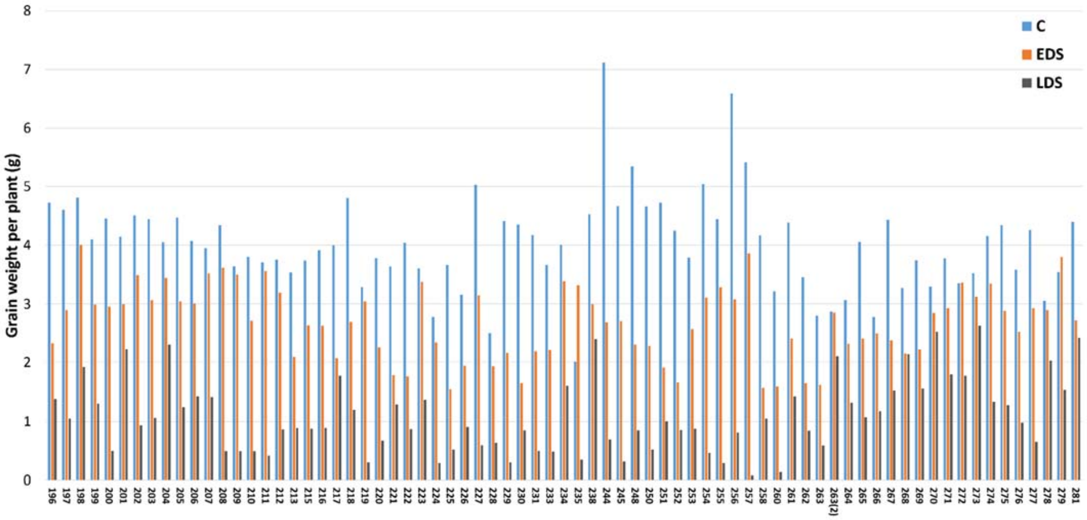
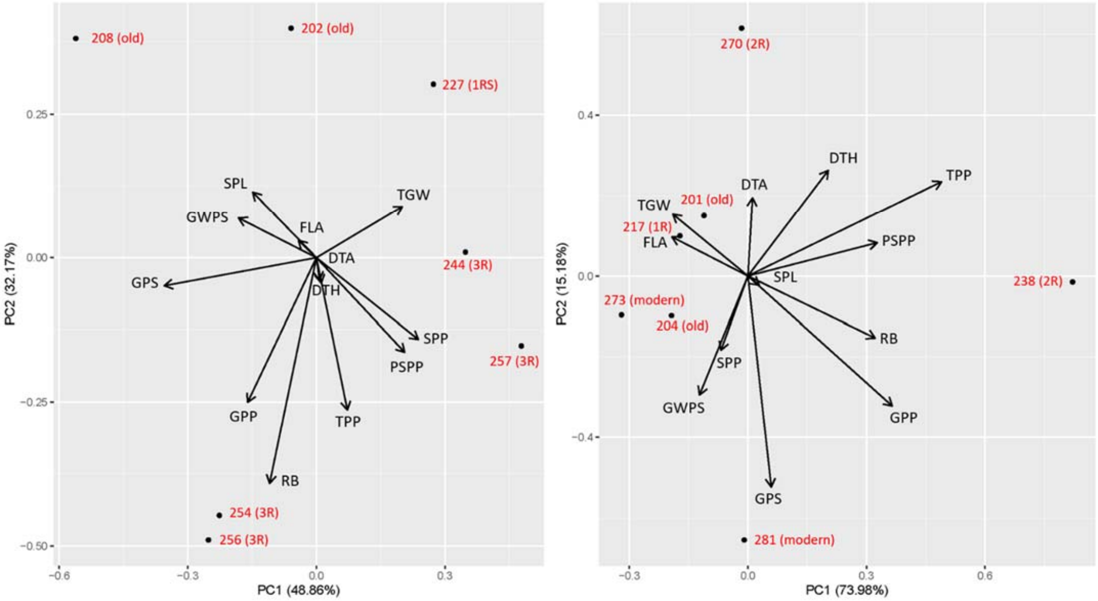


Figure 5. Yield (grain weight per plant = GWPP) of each genotype under control (C), early drought stress (EDS) and late drought stress (LDS).

Based on the STI values of GWPP, the genotypes 257 (3R), 256 (3R), 244 (3R), 227 (1RS), 202 (old), 208 (old) and 254 (3R) were found to be the top 10% genotypes under EDS with STI values ranging from 0.97 to 1.29, and the genotypes 238 (2R), 281 (modern), 204 (old), 273 (modern), 201 (old), 270 (2R) and 217 (1R) were found to be the top 10% genotypes under LDS with STI values ranging from 0.44 to 0.67 (Table 1). From the PCA with all the studied traits except GWPP, it was clear that different traits contributed to the high performance in terms of GWPP in the top 10% of genotypes. Under EDS, GPP and RB contributed to the highest extent to GWPP in the genotypes 254 (3R) and 256 (3R), while TGW and SPP had the highest impact on GWPP in the genotypes 227 (1RS) and 257 (3R), respectively (Figure 6a). Under LDS, TGW and FLA positively contributed to GWPP while GPP and RB showed a negative impact on GWPP in the genotypes 201 (old) and 217 (1R). On the contrary, a positive impact on GWPP was found with PSPP and RB in the genotype 238 (2R) (Figure 6b).

Table 1. Top 10% genotypes selected by stress-tolerance index (STI) of yield (grain weight per plant = GWPP) under early drought stress (EDS) and late drought stress (LDS).

EDS			LDS		
Genotype	**STI**	**Genetic Background**	**Genotype**	**STI**	**Genetic Background**
257	1.29	3R	238	0.67	2R
256	1.25	3R	281	0.66	modern
244	1.18	3R	204	0.58	old
227	0.98	1RS	273	0.57	modern
202	0.97	old	201	0.57	old
208	0.97	old	270	0.51	2R
254	0.97	3R	217	0.44	1R



(a) (b)

Figure 6. Principal-component analysis (PCA) for stress-tolerance index (STI) of all traits except grain weight per plant (GWPP) with the top 10% genotypes selected by stress-tolerance index (STI) of GWPP under (**a**) early drought stress (EDS) and (**b**) late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, Old = cultivars released from 1928 to 1990, 1R = introgressions of chromosome 1R, 1RS = introgressions of chromosome 1RS, 2R = introgressions of chromosome 2R, 3R = introgressions of chromosome 3R.

3. Discussion

Corresponding to the hypothesis of the present study, drought tolerance at various genotypes and genotype groups were investigated, representing different genetical constitutions. Thereby, the genetic background of the drought tolerance could be identified. Thus, old genotypes and alien wheat lines with 1R or 1RS showed a robust early root growth, which is most likely a characteristic of importance for drought-stress tolerance. However, the old genotypes and the lines with chromosome 1R and 1RS did not result in a high final-yield performance after drought stresses. Instead, the genotypes with 3R were found to be among the top 10% of genotypes that are tolerant to EDS while no clear pattern of the dominant genetic background was shown among the 10% best performing genotypes under LDS. Interestingly, separate genetic mechanisms for early root and shoot growth were demonstrated in the present study. Furthermore, our results demonstrated that RB, GPP and SPP were important characteristics that correlated with tolerance to EDS while TGW and FLA correlated positively with tolerance to LDS. This finding suggested that the general yield-based drought tolerance of wheat could be decomposed into multiple specific traits that potentially contribute to the high performance of the yield under drought.

A significantly higher early root growth, here measured as DRL, was found in the old wheat genotypes and alien genotypes with chromosome 1R and 1RS, as compared to modern wheat and wheat with other alien introgressions. Thus, genes contributing to early root growth seem to be present both in the wheat material consisting of old Swedish wheat lines and on chromosome 1RS. Previous studies have shown that an extensive root system in wheat enhances water and nitrogen capture, thereby explaining that a vigourous root system contributes to the yield performance of wheat under water deficiency [32].

An early root growth might be a trait of significant relevance for a good performance during drought stress, as early root growth might contribute to a higher chance of reaching the humidity during dry conditions [33]. A general understanding among Swedish growers is that old wheat varieties have a strong root growth, which contributes to their good performance during dry conditions, although scientific evidence for such a statement is lacking. However, in a recent study, a larger root system was observed in old wheat varieties than in modern wheat [34]. Furthermore, this large-to-small change in root biomass from old to modern wheat has been confirmed by several studies [35–37]. The relatively small root system in modern wheat could be attributed to the years of recurrent breeding programs that were mainly aimed at increasing the aboveground biomass, and especially the grain yield. Hence, the biomass shift from underground to aboveground was established along with years of yield-oriented selection.

The findings of increased early root vigour in the genotypes with 1R and 1RS introgressions in the present study correspond well with the findings of other studies as reported below. Several studies have indicated positive performance at abiotic and biotic stress conditions by the introgression of 1RS in wheat [26]. The 1RS has been reported to confer disease resistance to wheat. For example, the resistance genes *Sr31*, *Yr9*, *Lr26* and *Pm8* were reported to be introduced to wheat along with chromosome 1RS, and effectively resisted or mitigated stem rust, stripe rust, leaf rust and powdery mildew, respectively [38,39]. A previous study using molecular markers provided evidence for the presence of *Sr31* in 1BL.1RS translocation lines [40]. Improved resistance to stripe rust and powdery mildew was reported in five 1BL.1RS translocation lines [41] and a newly detected gene, *Sr59* introgressed to wheat from 2R, has been reported to confer resistance to all currently known stem-rust races [29]. Translocations of 1RS to the long arm of wheat chromosome 1 (1AL, 1BL and 1DL) were shown to increase root biomass and yield in spring wheat [42]. The gene(s) for rooting ability have been suggested to be present in the distal 15% of the physical length of the 1RS arm [31]. Recently, several studies have provided evidence that the 1RS translocation improves the root traits of wheat [43–45]. In addition to the improvement of root traits, the 1RS translocation has been reported to contribute to increased grain yield [41,46,47]. Despite all the positive effects from introgression of the chromosome 1RS, grains of introgression lines containing 1RS were found to exhibit bread-making quality defects including sticky dough. The reduction in bread-making quality by 1RS has been related to the presence of the rye storage protein, secalin, which is encoded by *Sec-1*, and to a decrease in dough strength due to the loss of *Glu-B3/Gli-B1*-encoding gliadins and low-molecular-weight glutenins [48–51].

The results of the present study showed that both DRL and DLA varied significantly among genotypes, but a correlation between DRL and DLA was lacking. These findings indicate that early root and shoot growth of wheat are regulated by separate genetic mechanisms, which could possibly be either different genes or the same genes with different expression timings. However, previous studies have indicated a strong association between root and shoot traits at maturity [52–54], and old genotypes are reported to have both higher root and shoot biomass than modern genotypes [55,56]. Also, several dwarfing genes e.g., *Rht-B1c*, *Rht-D1c* and *Rht12* were found to significantly affect the root length of wheat at early stages [57]. Other studies have shown that a class of *Rht* genes did not affect shoot-growth traits such as coleoptile length, leaf-elongation rate and responsiveness to Gibberellin at early stages [58]. Another study showed that significant root-biomass differences observed at early vegetative stages among five wheat genotypes were clearly reflected in the leaf area and leaf biomass at later stages [59], which indicated the correlation between early root vigour and late shoot traits. Despite the above-mentioned findings, the relationships among root and shoot characteristics at early crop growth and the effects on the mature crop are still uncertain and need further evaluation.

The layout of the Biotron experiment under controlled conditions allowed us to selectively treat the plants with drought stress, explicitly avoiding other environmental variations, and to observe the variation of each of the 13 selected traits among the genotypes.

Unexpectedly, the early root vigour of the old genotypes and the lines with 1RS did not contribute to the drought tolerance of these genotypes at maturity. We attribute the lack of correlation between early root vigour and drought stress to the fact that the experiment was carried out in pots in an indoor controlled-environment space, where a larger and more robust root system does not play a role in the opportunity to reach the humidity and nutritional elements. Field conditions provide considerably more space for the root system to expand in order to reach water and nutrient sources. From reported results based on wheat plants grown in a sufficiently large space, e.g., 1 m and 1.5 m PVC tubes [35] and 1.6 m columns [60], old wheat genotypes did show higher root biomass and longer roots than modern genotypes at maturity. Similarly, increased root biomass in wheat-rye introgression lines with chromosomes 1R and 1RS was noted by studies that were carried out in field conditions [43,44]. Long-rooting genotypes grown in conditions where the spread of their root systems have an impact on reaching water and nutrients should most likely be more drought tolerant than genotypes with smaller and less spread root systems. Studies in real field conditions are needed to evaluate the effect of the superior root system of old genotypes and the genotypes with chromosome 1R and 1RS on drought tolerance.

Our results suggest that 3R might be useful to improve early drought tolerance in wheat, which is an increasingly important characteristic for Nordic conditions, as well as under the predicted climate-change conditions. Differences in early drought tolerance were noted among the genotype groups evaluated here with the least change in yield (a high STI of GWPP) by the early drought treatment in the genotypes containing 3R. Additionally, four (257, 256, 244 and 254) of the top 10% of genotypes were identified as containing 3R. Previous studies on drought tolerance in wheat showed limited findings on stress-tolerance genes connected to chromosome 3R. To our knowledge, there is just one field study that proposes that genes regulating drought tolerance are present on chromosomes 7R, 3R and 5R [61]. Instead, 3R, along with 4R, 6R and 7R, has been well demonstrated as one of the rye chromosomes carrying major genes for the tolerance to aluminium toxicity [62–66]. Our results, indicating genes for drought tolerance on 3R, were achieved under EDS, differently to most other studies on drought tolerance in wheat-rye introgressions, which have mainly been based on drought stress imposed at late stages [12,42,67]. Thus, the timing of the drought stress might be the reason for the lack of data in the literature on drought-tolerance genes on 3R, and the potential tolerance genes on 3R might be specific to early drought. The PCA result based on the top 10% of genotypes for EDS suggested that a large root system, together with high numbers of grains and spikes per plant as beneficial traits for high tolerance to early drought. Thus, the 3R chromosome seemed to contribute to the ability for the plant to set roots and spikes regardless of whether there had been an early drought period, which is a characteristic that might be of importance for the future climate change in the Nordic countries, where early drought seems to be an increasingly common feature.

The present study clearly indicated different mechanisms of wheat to combat to drought stress at different maturation stages. This was verified by the fact that the genotype groups with early root vigour and EDS tolerance were clearly distinguishable, while no clear pattern of LDS tolerance in a specific genotype group was detected in the present study. As anthesis is a critical period for grain formation in wheat, with a high impact on meiosis from drought [68], large impacts on final yield have often been described as a result of late drought [8,69]. The potential of the grain weight of wheat at maturity is closely determined by the number of endosperm cells per grain and the process of cell division in the endosperm, which ends within two to three weeks after anthesis [70]. Another factor determining the grain weight is cell expansion, which was found to be related to water content during the grain-filling period [71]. Drought stress at anthesis has been reported to shorten the grain-filling period of wheat [72], and the termination of cell expansion predetermines the maturation of grains [73,74]. Thus, the water deficit during this period is known to cause a severe reduction in grain yield. Unlike the direct and severe effects of post-anthesis drought on the final yield, the effects of early drought at the vegetative growth stages are more morphology-related, e.g., EDS affects plant height, leaf area and number of tillers [75]. In the present study, the PCA results based on the top 10% of genotypes for LDS indicated that TGW and FLA might account for the high tolerance of genotypes 201 (old) and 217 (1R), while RB and GPP showed a negative correlation to the high tolerance. Hence, large grain size and flag leaves are beneficial traits for high tolerance to late drought, and the negative contribution of RB and GPP to the high tolerance to late drought further confirmed the different mechanisms of wheat plants to combat early and late drought.

Yield performance under drought stress is the major concern when breeders develop drought-tolerant crop varieties [76] because, in a broad sense, drought tolerance is defined as the yield-maintaining ability of a plant under drought. The empirical approach used in breeding programs for drought-tolerant genotypes emphasizes the yield under both non-stressed and stressed conditions, so that both yield stability and yield potential can be evaluated [77]. However, the complexity of drought tolerance has been widely accepted, particularly from a genomics perspective, due to the identification of many drought-responsive genes [78]. The *Lea* genes encoding late-embryogenesis-abundant (LEA) proteins were reported to be highly related to drought tolerance [79–81]. Another large drought-related gene family is the *NAC* genes that encode transcription factors. By introducing a rice *NAC1* gene (*SNAC1*) into a wheat variety, significantly enhanced drought tolerance was achieved in the transgenic wheat plants [82]. The V-PPase gene family that encodes an enzyme vacuolar H+-translocating pyrophosphatase was found to be responsive to abiotic stress. Improved drought tolerance was observed in transgenic *Arabidopsis* plants that were overexpressing a V-PPase member of wheat *TaVP1* [83]. Therefore, due to the polygenic nature of drought tolerance, using yield as the only selection criterion to screen for a drought-tolerant genotype might not be the optimal approach. Valuable information on drought tolerance that is hidden behind other traits could be missed. However, blindly including too many traits into a selection process could result in an unnecessary increase in workload and provide a misleading result. As demonstrated earlier in this paper, we propose that the yield-based drought tolerance of wheat can be explained by other traits, and therefore identifying and combining the most yield-contributing traits during selection may help achieve more effective results than selecting based on yield alone.

4. Materials and Methods

4.1. Plant Materials

A collection of 73 spring-wheat (*Triticum aestivum*) genotypes consisting of 14 Swedish landraces and old cultivars released from 1928 to 1990 [84], 50 wheat-alien introgression lines [85] with rye chromosomes 1R, 2R, 3R, 4R, 5R, 6R and Leymus Racemosus chromosome N in the form of translocation and substitution [29,40], and nine modern wheat breeding lines from the company Lantmännen were used in this study (Table S5). All the wheat lines with different genetic backgrounds (old, modern, introgression lines) will be referred to as genotypes from here and onwards in this publication.

4.2. Early Root and Shoot Phenotyping

To evaluate the early root growth of the genotypes, a hydroponic experiment was conducted in the greenhouse, germinating the wheat seeds on wet filter paper at low-temperature conditions (4 °C, 48 h) for uniform germination. Following previously described methods [13,86], germinated seeds were fixed on dedicated blue blotter paper (210 mm × 297 mm, Anchor paper company) with small clips and covered by black plastics. The wetness of the paper was maintained by dipping its bottom in water. After seven days of growth under room temperature (25 °C/18 °C day/night), root imaging was performed with a DSLR camera (Canon 1300D, Canon Inc., Tokyo, Japan) mounted on a kaiser stand 40 cm above the root surface. The image-based digital-root length (DRL) was extracted using the software RootNav [87].

To evaluate the early shoot growth, the genotypes were phenotyped in the Biotron (growing conditions described below) from three different angles using two digital singlelens reflex (DSLR) cameras (Canon 1300D, Canon, USA) 20 days after sowing as previously described [88]. Cameras were operated through the software digiCamControl (http://digicamcontrol.com/, accessed on 28 February. 2020). The image-based digital-leaf area (DLA) was extracted using the software Easy Leaf Area (https://www.quantitative-plant.org/software/easy-leaf-area, accessed on 28 April 2020).

Comparisons of DLA and DRL were made on an individual-genotype basis, but groups of genotypes were also compared (i.e., modern wheat genotypes, old Swedish genotypes, 1R wheat-alien introgression lines, 1BL.1RS wheat-alien translocation lines, 2R wheat-alien introgression lines, 3R wheat-alien introgression lines).

4.3. Biotron Trial

The experiment was carried out in 2020, growing the genotypes from April–September in a daylight (DK) chamber with natural light in the Biotron at the Swedish University of Agricultural Sciences in Alnarp, Sweden. The temperature and humidity were strictly regulated hourly based on the mean weather data of Malmö over the past decade (2010–2019). The data were obtained from Swedish Meteorological and Hydrological Institute (SMHI) (Table S6). Five seeds of each genotype and for each treatment were sown in each 2.5 L plastic pot filled with peat-based soil, and after thinning, three plants were retained in each pot. Thus, three biological replicates of each genotype were subjected to each of the three growing conditions, i.e., standard growing conditions used as a control (C), early-drought-stress (EDS) growing conditions, and late-drought-stress (LDS) growing conditions. To achieve uniform solar irradiance for growth throughout the experiment, the position of each pot was shifted within the chamber weekly.

4.4. Growing Conditions including Drought Stress

For the C treatment, the plants were watered every second day so that the soil moisture was maintained throughout the whole cultivation period and watering was stopped when the spikes turned yellowish in order for the plants to become mature. The EDS-treated plants were watered similarly to the C plants until day 30 (Zadoks 23) after planting [89] when the drought treatment started, by withholding water for 28 days, and thereafter the watering again followed the C treatment. Similarly, the LDS plants followed the C treatment until day 60 (beginning of heading stage, Zadoks 50) after planting, when water was withheld for 14 days, and thereafter the C treatment started again. The soil moisture on the last day of both drought treatments was below 1%, as measured by a moisture meter (Takemura electric works DM-15 soil PH & moisture tester hygrometer).

4.5. Morphological, Phenological and Yield Traits

Days to heading (DTH) (number of days taken from sowing to appearance of spikes) and anthesis (DTA) (number of days taken from sowing to appearance of anthers) were recorded manually. Spike length (SPL) was measured with a ruler in centimetres. Tillers per plant (TPP), spikes per plant (SPP), productive spikes per plant (PSPP), grains per plant (GPP) and grains per spike (GPS) were counted. Flag-leaf area (FLA) [90] was measured with an LI-3000C Portable Leaf Area Meter (LI-COR Environmental). Grain weight per plant (GWPP), grain weight per spike (GWPS), 1000-grain weight (TGW) (CONTADOR seed counter, PFEUFFER, Kitzingen, Germany) and dry root biomass (RB) were measured in grams. Thus, yield per plant = GWPP.

4.6. Data Analysis

The stress-tolerance index of five yield traits, including GWPP, GWPS, GPP, GPS, and TGW, was calculated to determine the comprehensive responses of genotypes to drought stresses by using the following formula.

Stress-tolerance index (STI) [15]:

$$\mathrm{STI} = (\mathrm{Ys} \times \mathrm{Yp})/(\overline{\mathrm{Yp}})^2, \quad (1)$$

where Ys represents the yield trait of each genotype under drought-stress conditions; Yp and $\overline{Yp}$ represent the yield trait of each genotype and the mean yield trait of all genotypes in the productive conditions, respectively.

All the statistical analyses were performed using software RStudio [91], version 1.2.5042. A two-way ANOVA was conducted for each trait to detect significant differences between the treatments. Where significant differences ($p < 0.05$) were detected, multiple mean comparisons were conducted using the Tukey post-hoc test with the package rstatix. Pearson's correlation coefficients were calculated to investigate correlations among different traits with their mean values using the package Hmisc, and the results were visualized using the package corrplot. Principal-component analysis (PCA) was performed with the package ggfortify to further explore the relationship among different drought-related traits and treatments.

5. Conclusions

Global food security is currently threatened by dynamic climate changes and the growing global population, leading to an increase both in abiotic stress conditions and in demands. To cope with this situation, and with the loss of genetic diversity in modern breeding lines over the last century, diverse genetic materials are untapped resources with which to search for candidate genes that contribute to high yield under stress conditions. Early root vigour is a useful characteristic for the plant to sustain stressful growing conditions, e.g., from drought, which is present in the old Swedish lines and lines with 1R and 1RS. The early root vigour in such lines is not necessarily correlated to early shoot growth, indicating the possibility of different genetic determinants of these characteristics. The 3R may contain important genes contributing to tolerance in wheat for early drought stress, which is a characteristic already of importance under Nordic conditions, although early drought tolerance is expected to become even more important with the predicted climate change. The mapping of the responsible genes and the plant traits determining early drought tolerance is therefore an important challenge. Here, grains per plant, root biomass and spikes per plant were the dominating traits that contributed to a low change in yield in the genotypes with good tolerance to early drought. This indicated the ability to grow and set seeds despite an early drought period as an important property of early drought tolerance. Differently, the 1000-grain weight and a large flag-leaf area were the most important traits for a low change in yield and therefore a good tolerance to late drought. Thus, breeding targets related to early vigour or breeding for drought resistance at various developmental stages need to be set, and suitable genes need to be determined and mapped in order to successfully breed drought-tolerant spring wheat.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23063333/s1.

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Diverse wheat lines to mitigate the effect of drought on end-use quality

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Global climate change is causing an increasing number of drought events, which might impact the stability of wheat breadmaking quality. In this study, 73 spring wheat lines with diverse genetic backgrounds (modern, old, and wheat–rye introgression) were drought treated, and the grains were analyzed by high-performance liquid chromatography for protein composition traits related to breadmaking quality. The amount of total sodium dodecyl sulfate-extractable and -unextractable proteins (TOTE, which correlates to grain protein content) increased significantly under late drought, while no effect of early drought was found on the analyzed protein composition traits. Under control treatment, genotypes with 3R showed significantly higher TOTE than genotypes with 1R, 1RS, and 2R, indicating the potential role of 3R in increasing grain protein concentration. The lower percentage of sodium dodecyl sulfate-unextractable polymeric protein in the total polymeric protein (%UPP) found in 1R and 1RS genotypes as compared to modern and old genotypes suggested a gluten strength reduction induced by 1R and 1RS. Despite the negative yield–protein correlation found in this study, lines 252 (3R), 253 (3R), and 258 (2R) displayed the presence of germplasm with both high yield and protein concentration. The %UPP was found to be positively correlated to spike-size-related traits (grains per spike, grain weight per spike, and spike length) across all three treatments. Additionally, high and stable TOTE was mainly obtained in genotypes with 3R, while old genotypes showed dominant performance in %UPP. Thus, genes responsible for high and stable protein concentration and gluten strength should be explicitly searched among introgression lines with chromosome 3R and old Swedish cultivars, respectively.

KEYWORDS

protein composition, protein concentration, gluten strength, drought, yield, wheat

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Abbreviations: C, control treatment; EDS, early drought treatment; LDS, late drought treatment; LPP, large polymeric proteins; SPP, smaller polymeric protein; LMP, large monomeric protein; SMP, smaller monomeric protein; TOTE, total SDS-extractable protein; TOTU, total SDS-unextractable protein; %UPP, the percentage of SDS-unextractable polymeric protein in the total polymeric protein; %LargeUPP, the percentage of large SDS-unextractable polymeric protein in the total large polymeric protein; %LUMP, the percentage of large SDS-unextractable monomeric protein in the total large monomeric protein; Mon/Pol, the ratio of total monomeric protein to the total polymeric protein; SNPP, spike number per plant; SPL, spike length; PSPP, productive spikes per plant; TGW, 1000-grain weight; GPS, grains per spike; GWPS, grain weight per spike; GWPP, grain weight per plant; and GPP, grains per plant.

1 Introduction

With climate change, the global average temperature has increased over the decades. It is predicted to increase even faster in the future (Allen et al., 2019), thereby resulting in increased frequency and length of droughts. Drought has been reported as the major limiting factor to crop production as it significantly restricts plant growth and impacts the physiological, biochemical, morphological, and molecular characteristics of plants (Gregorova et al., 2015; Chaudhry and Sidhu, 2022). Thus, significant drought-induced yield losses have been reported from different regions of the world (Fischer and Maurer, 1978; Giunta et al., 1993; Senapati et al., 2019). Although grain yield is generally considered the most important trait, the end-use quality of certain crops is directly linked to processing efficiency, and drought-related effects on such parameters are also of relevance (Li et al., 2013).

Wheat (*Triticum aestivum*) is one of the three major crops worldwide and, thus, necessary in the human diet as a source of calories and proteins (Shiferaw et al., 2013). The breadmaking quality of wheat is an essential end-use character, which has been found to be highly correlated to protein composition (Branlard and Dardevet, 1985; Payne et al., 1987; Park et al., 2006). Proteins present in the wheat grain are divided into albumins, globulins, gliadins, and glutenins based on their solubility (Osborne, 1907). The gliadins and glutenins, also determined as gluten proteins, comprise 85% of the wheat grain proteins. The gluten proteins form polymers and monomers in the wheat grain (Markgren et al., 2020), which are reformed and rearranged during processing (Johansson et al., 2013). The specific distribution of polymeric and monomeric proteins determines the flour properties, including breadmaking quality (Gupta et al., 1993; Gupta et al., 1996; Zhang et al., 2008; Johansson et al., 2013). In addition, the polymerization behavior of the proteins is known to affect the breadmaking quality; e.g., the unextractable polymeric protein in total polymeric protein (%UPP) correlates positively with gluten strength (Gupta et al., 1993; Jia et al., 1996), while the total amount of SDS-extractable proteins (TOTE) correlates positively with the grain protein content (Johansson et al., 2013). Therefore, %UPP and TOTE are the two main protein parameters determining the end-use quality of the wheat grain.

Wheat protein composition is a grain quality property that varies among genotypes with different genetic backgrounds (Shewry et al., 1994; Veraverbeke and Delcour, 2002; Johansson et al., 2013). Compared to modern wheat, old wheat has a higher ratio of gliadins to glutenins, which contributes to weaker gluten, being less suitable for modern bread-baking processes (Desheva et al., 2014; Geisslitz et al., 2019). The introgression of the rye chromosome 1RS to the wheat genome has, in several previous studies, been reported to reduce the breadmaking quality by producing dough with unusually high stickiness and low strength (Dhaliwal et al., 1987; Graybosch et al., 1993; Fenn et al., 1994), even though the introgression of 1RS brought improvements in yield and disease resistance (Kim et al., 2004; Purnhauser et al., 2011; Ren et al., 2018).

In addition, environmental factors such as temperature, nitrogen, and soil moisture have been reported to contribute to differences in protein concentration and composition (Graybosch et al., 1995; Johansson et al., 2013; Rozbicki et al., 2015). Both grain protein concentration and bread loaf volume have been reported to increase under drought treatment (Kimball et al., 2001; Guzmán et al., 2016), while increases in %UPP and TOTE were noted under drought at the heading stage (Leiva et al., 2021). Furthermore, prolonged heat and drought in 2018 resulted in an increase in % UPP, though with a decrease in protein content, compared to the data from 2017 in Sweden (Lama, 2023). Differences in gluten strength were found as a result of variations in precipitation and temperature during the crop season (Johansson et al., 2020; Mkhabela et al., 2022). Although several observations have been made evaluating the effects of drought on wheat gluten, more studies are urgently needed to deepen our understanding of the relationship between yield performance, protein composition and drought stress in the context of fast global climate change.

The present study aimed to evaluate the effects of early and late drought on protein content and composition in a wide variety of spring wheat genotypes. Changes in different protein parameters were related to the diverse genetic background of the wheat evaluated, i.e., the modern, old, and wheat–rye introgression lines. Furthermore, previously obtained yield data were included in this study to establish the connection between drought effects on grain yield and quality. This entire study was designed based on the hypothesis that genes from different genetic sources impact baking quality performance under drought stress.

2 Materials and methods

2.1 Plant materials

A total of 73 spring wheat (*T. aestivum* L.) genotypes with a wide genetic background (Lan et al., 2022) consisting of 9 modern wheat cultivars and breeding lines received from the breeding company Lantmännen, 14 Swedish old cultivars released between 1928 and 1990 (Johansson et al., 2021), and 50 wheat-alien introgression lines (Merker, 1984) with rye chromosomes 1R, 2R, 3R, 4R, 5R, 6R, and *Leymus racemosus* (wild rye) chromosome N in the form of translocation and substitution (Rahmatov, 2016) were used in this study.

2.2 Growing conditions and drought treatments

All grain samples were collected from an experiment carried out in 2020 under strictly controlled conditions in the Biotron facility at the Swedish University of Agricultural Sciences, Alnarp, Sweden, as has previously been described (Lan et al., 2022). Thus, the hourly regulated temperature and humidity were set according to the mean climate data of Malmö, Sweden, from 2010–2019, obtained from the Swedish Meteorological and Hydrological Institute (SMHI). Three growing conditions were implemented for all the genotypes, i.e., standard growing conditions used as a control (C) where plants were watered every second day throughout the experiment, early drought stress (EDS) growing conditions where a 28-day water withholding was imposed from 30 days after planting, and late drought stress (LDS) growing conditions where a 14-day water withholding was imposed from 60 days after planting. Details of the temperature, humidity, and treatments for the present study are as previously described by Lan et al. (2022).

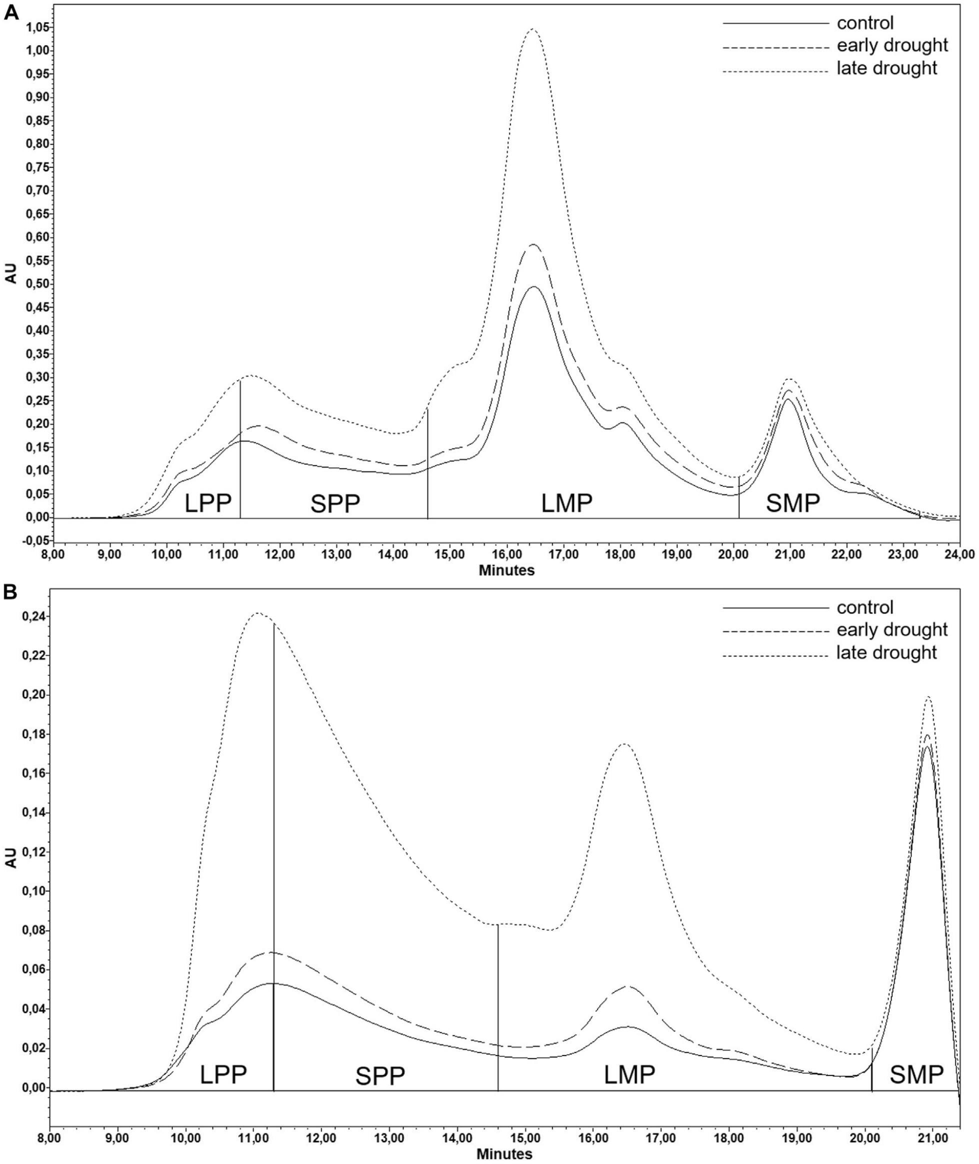

FIGURE 1
Example (introgression genotype 256, chromosome: 3R) of SE-HPLC chromatograms of **(A)** SDS-extractable and **(B)** SDS-unextractable gluten proteins under different treatments. Chromatograms were divided into four sections representing large polymeric proteins, smaller polymeric proteins, large monomeric proteins, and smaller monomeric proteins. The area of each section under the chromatograms represents the amount of the corresponding protein fraction. AU, absorbance unit of the UV detector.

2.3 Grain protein content

The protein content of flour samples (73 × 3 biological replicates) harvested from the control condition was determined using the elemental particle analyzer method (EPA, Flash 2000 Elemental Analyzer, Thermo Scientific). A conversion factor of 5.7 was used to convert the nitrogen content into protein content (Mosse, 1990).

2.4 Size-exclusion HPLC

For each genotype (total of 73), spikes of three biological replicates for each growing condition were sampled, resulting in a total of 657 samples. After threshing, the husked grains of each genotype were milled to flour for 30 s by using a laboratory mill (Mixer Mill 400 MM, RETSCH) to obtain the whole-grain flour samples.

Extraction of proteins was carried out on two technical replicates of each of the 657 whole-grain flour samples, following a previously developed two-step protocol (Gupta et al., 1993) with minor modifications. In the first step, 16.5 mg of each flour sample was used to extract proteins with 1.4 ml of 0.5% SDS-phosphate buffer (pH 6.9). The samples were subjected to 10 s of vortexing (VOTEX-GENIE 2, Scientific Industries) followed by 5 min of stirring (VIBRAX, VXR basic, IKA) at 2,000 rpm and centrifugation (LEGEND MICRO 17, Thermo Scientific) for 30 min at 10,000 rpm. According to the work of Larroque et al. (2000), the supernatant was subjected to a 2 min water bath at 80°C (SUB Aqua Pro, Grant) to avoid degradation of polymeric proteins by deactivating the enzyme protease followed by a 2 min ice-water bath to cool the supernatant down to a suitable status for SE-HPLC. In the second step, 1.4 ml of 0.5% SDS-phosphate buffer (pH 6.9) was added to each of the residual pellet from the first extraction, which were then subjected to sonication for 45 s in an ultrasonic disintegrator (Soniprep 150, Tamro, Mölndal, Sweden) with a 3 mm exponential microtip at an amplitude of 5 µm. After sonication, all the samples were centrifuged for 30 min at 10,000 rpm, the supernatant of each sample was collected, and following the work of Larroque et al. (2000), it was treated for 2 min at 80°C and then in an ice-water bath.

All samples (total 2628) from the two-step extraction procedure were loaded onto a Waters (Milford, MA, United States) HPLC system with a BioSep SEC-4000 Phenomenex column. Each sample was separated according to molecular size distribution under 30 min, and the proteins were detected by using a diode array detector at a UV absorbance of 210 nm.

The results of SE-HPLC were output in the form of two chromatograms (Figures 1A, B), one from the first extraction and one from the second extraction. The wheat proteins consist of gliadins, which are monomeric in their native form, and glutenins, which are polymeric in their native form (Markgren et al., 2020). However, during any kind of processing, cross-links are broken and reformed among the proteins, which results in gliadins being trapped (Johansson et al., 2013) or cross-linked through disulfide, peptide, or lanthionine bonds or through hydrophobic clustering into the polymer (Markgren et al., 2022). Extraction of the proteins using SDS-phosphate buffer for the first step and sonication for the second step is also known to not break all of the disulfide bonds of the polymers (Johansson et al., 2013). Therefore, both of the chromatograms visualized the presence of both polymeric and monomeric proteins of various sizes, which has previously been described in various studies (Gupta et al., 1993; Johansson et al., 2013). Therefore, both chromatograms of each sample were divided into four sections according to molecular size, namely, large polymeric proteins (LPP), smaller polymeric proteins (SPP), large monomeric proteins (LMP), and smaller monomeric proteins (SMP). The relative amounts of these four types of proteins were calculated based on areas covered by the absorbance curve (Malik, 2012). Six protein parameters (Malik, 2012), including total SDS-extractable protein, total SDS-unextractable protein (TOTU), the percentage of SDS-unextractable polymeric protein in the total polymeric protein (%UPP), the percentage of large SDS-unextractable polymeric protein in the total large polymeric protein (%LargeUPP), the percentage of large SDS-unextractable monomeric protein in the total large monomeric protein (%LUMP), and the ratio of total monomeric protein to the total polymeric protein (Mon/Pol), were calculated as follows:

$$\mathbf{TOTE} = \mathbf{eLPP} + \mathbf{eSPP} + \mathbf{eLMP} + \mathbf{eSMP},$$

$$\mathbf{TOTU} = \mathbf{uLPP} + \mathbf{uSPP} + \mathbf{uLMP} + \mathbf{uSMP},$$

$$\mathbf{\%UPP} = (\mathbf{uLPP} + \mathbf{uSPP}) \times \mathbf{100} / (\mathbf{eLPP} + \mathbf{eSPP} + \mathbf{uLPP} + \mathbf{uSPP}),$$

$$\mathbf{\%LargeUPP} = \mathbf{uLPP} \times \mathbf{100} / (\mathbf{eLPP} + \mathbf{uLPP}),$$

$$\mathbf{\%LUMP} = \mathbf{uLMP} \times \mathbf{100} / (\mathbf{eLMP} + \mathbf{uLMP}),$$

$$\mathbf{Mon/Pol} = (\mathbf{eLMP} + \mathbf{eSMP} + \mathbf{uLMP} + \mathbf{uSMP}) / (\mathbf{eLPP} + \mathbf{eSPP} + \mathbf{uLPP} + \mathbf{uSPP}).$$

Here, ‘e’ and ‘u’ represent SDS-extractable and SDS-unextractable proteins, respectively. Based on previous results, TOTE was used in the present study as an indicator of grain protein content, and similarly, %UPP was used as an indicator of gluten strength (Malik, 2012). To verify the strong correlation between TOTE and grain protein content, a correlation analysis between the two parameters was carried out as shown in the appendix (Supplementary Figure S1).

2.5 Plant physiological yield traits

Yield-related traits obtained from the physiological development of the plant, such as spike length (SPL), spike number per plant (SNPP), productive spikes per plant (PSPP), 1000-grain weight (TGW), grains per spike (GPS), grain weight per spike (GWPS), grain weight per plant (GWPP), and grains per plant (GPP), were reported in a previous study (Lan et al., 2022). Here, we evaluated the relationship between these yield traits and the grain protein content and composition obtained from the aforementioned HPLC analyses.

2.6 Data analysis

All the statistical analyses were carried out using the software RStudio (Team, 2015). A two-way analysis of variance (ANOVA) was conducted for each protein parameter to detect significant variations between treatments and among genotypes. A mean comparison was performed to verify the significant differences between treatments pairwise and compare different genotype groups (modern, old, 1R, 1RS, 2R, and 3R) using the LSD *post hoc* test with the R package “agricolae.” Pearson’s correlation coefficients between the six protein composition traits and eight previously obtained yield traits, i.e., SPL, SPP, PSPP, TGW, GPS, GWPS, GWPP, and GPP (Lan et al., 2022), were calculated and visualized using R packages “Hmisc” and “corrplot,” respectively. Principal component analysis (PCA) was computed and visualized

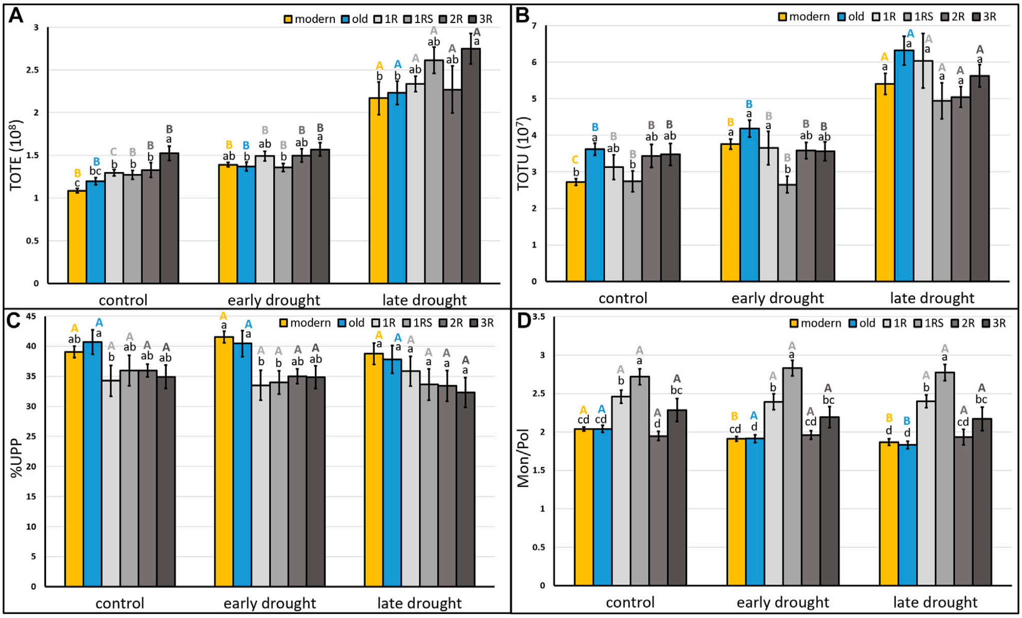

FIGURE 2
Mean **(A)** total SDS-extractable proteins, **(B)** total SDS-unextractable proteins, and **(C)** the percentage of SDS-unextractable polymeric proteins in the total polymeric proteins; **(D)** the ratio of total monomeric proteins to the total polymeric proteins of each genotype group under control, early drought, and late drought treatments. Modern = approved cultivars and breeding lines received from company Lantmännen. Old = cultivars released from 1928 to 1990. 1R = introgressions of chromosome 1R. 1RS = introgressions of chromosome 1RS. 2R = introgressions of chromosome 2R. 3R = introgressions of chromosome 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same lower letters do not differ significantly (LSD *post hoc* test at $p < 0.05$).

using the R package “ggfortify” to further study the relationship among different traits and treatments. The harmonic mean of genotypic values (HMGV), relative performance of genotypic values (RPGV), and harmonic mean of relative performance of genotypic values (HMRPGV) were computed based on the best linear unbiased prediction (BLUP) using the R package ‘metan’ to rank genotypes with the highest TOTE and %UPP stability. The additive main effects and multiplicative interaction (AMMI) was performed using the R package ‘metan’ to study the TOTE and % UPP stability of genotypes across treatments.

3 Results

3.1 Effect of drought stresses on protein fractions

The grain protein content of the C samples varied between 8% and 15% (Supplementary Table S2) and was found to significantly and strongly correlate with TOTE (Supplementary Figure S1). Therefore, the TOTE values were used to evaluate the effects of drought on grain protein content. The ANOVA showed a significant effect of drought stress on three of the studied protein parameters, i.e., TOTE, TOTU, and Mon/Pol (Supplementary Table S2). Significantly higher TOTE and TOTU levels were obtained for LDS than C plants (C: 1.32 × 10^8, LDS: 2.33 × 10^8 and C: 3.2 × 10^7, LDS: 5.5 × 10^7, respectively; Supplementary Table S3). Drought stress at different stages affected the mean of the six protein composition traits evaluated (TOTE, TOTU, %UPP, %LargeUPP, %LUMP, and Mon/Pol) differently among the genotype groups (modern, old, 1R, 1RS, 2R, and 3R; Figure 2; Supplementary Figure S2). Thus, both TOTE and TOTU were significantly higher in all the six genotype groups under LDS as compared to C (Figure 2A, B), and only two genotype groups (modern and old) showed a significantly lower Mon/Pol under LDS than C (Figure 2D). For %UPP, %LargeUPP, and %LUMP, no significant effects of drought treatments were noted among treatments, although significant differences were found among genotype groups (Figure 2C; Supplementary Figure S2).

A significantly higher TOTU was found for old genotypes than for modern genotypes under C (modern: 2.72 × $10^{7;}$ old: 3.61× 10^7), while a higher %LargeUPP was found for modern than old genotypes under EDS (modern: 48.03; old: 42.53) (Figure 2B; Supplementary Figure S2A). For the introgression genotypes, the 3R genotypes showed a significantly higher TOTE compared to 1R, 1RS, and 2R under C (Figure 2A). Furthermore, 1RS genotypes

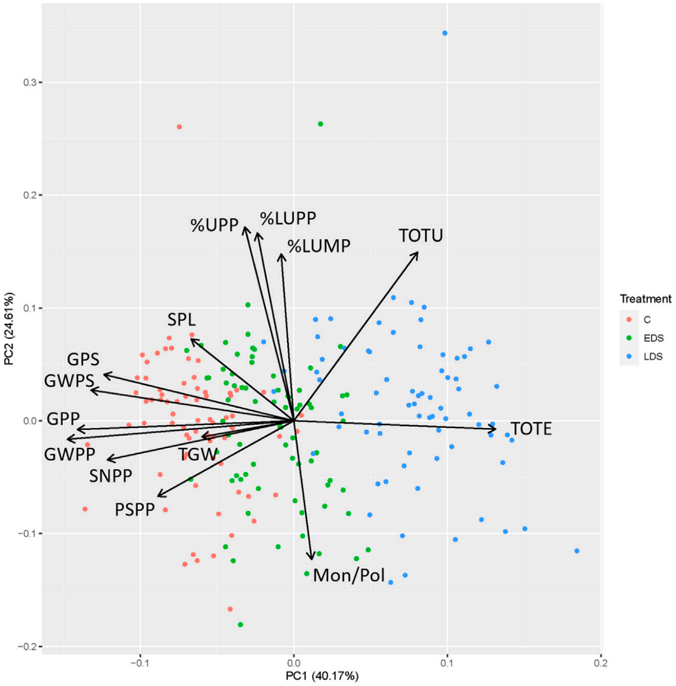

FIGURE 3
Biplot of principal component analysis for protein composition traits, total SDS-extractable proteins, total SDS-unextractable proteins, the percentage of SDS-unextractable polymeric proteins in the total polymeric proteins, the percentage of large SDS-unextractable polymeric proteins in the total large polymeric proteins, the percentage of large SDS-unextractable monomeric proteins in the total large monomeric proteins, the ratio of total monomeric proteins to the total polymeric proteins and yield traits, spike length, spike number per plant, productive spikes per plant, 1000-grain weight, grains per spike, grain weight per spike, grain weight per plant, and grains per plant of genotypes studied under control, early drought stress, and late drought stress.

showed a lower TOTU than 1R genotypes under EDS (Figure 2B). The 1R showed a higher %LUMP than 1RS under LDS (Supplementary Figure S2B). Significantly, the highest Mon/Pol was found for 1RS under all three treatments, and 1R genotypes showed a higher value than 2R genotypes (Figure 2D).

3.2 Relationships among treatments, protein composition, and yield traits

The PCA combining six protein composition traits (TOTE, TOTU, %UPP, %LargeUPP, %LUMP, and Mon/Pol) and eight previously obtained yield traits (SPL, SNPP, PSPP, TGW, GPS, GWPS, GWPP, and GPP) divided the samples based on drought treatment along the first principal component (PC1) axis, explaining 40.2% of the variation (Figure 3). Thus, C samples were located with negative values on the PC1, where the yield-related traits were found, indicating generally high values of these traits on the C samples. The LDS samples were found with basically positive values on the PC1, which also was the case for TOTE, indicating high grain protein concentration in the LDS samples. The second principal component (PC2), explaining 24.6% of the variation, generally differentiated the samples in relation to gluten-strength-related parameters (%UPP, %LargeUPP, %LUMP, and Mon/Pol), with a clear negative relationship between Mon/Pol versus %UPP, %LargeUPP, and %LUMP (Figure 3). Samples of all three treatments were differentiated along the PC2, thus indicating a variation in gluten strength in the plant material used independent of drought treatment.

3.3 Relationships between protein and yield traits

This study showed several significant correlations among the six protein composition traits (TOTE, TOTU, %UPP, %LargeUPP, %LUMP, and Mon/Pol) and eight yield traits, including three spike parameters (SPL, SNPP, and PSPP) and five grain-yield components (TGW, GPS, GWPS, GWPP, and GPP) under different treatments. A significantly negative correlation between TOTE and %UPP was noted and retained under C (−0.42***), EDS (−0.37**), and LDS (−0.49***; Figure 4).

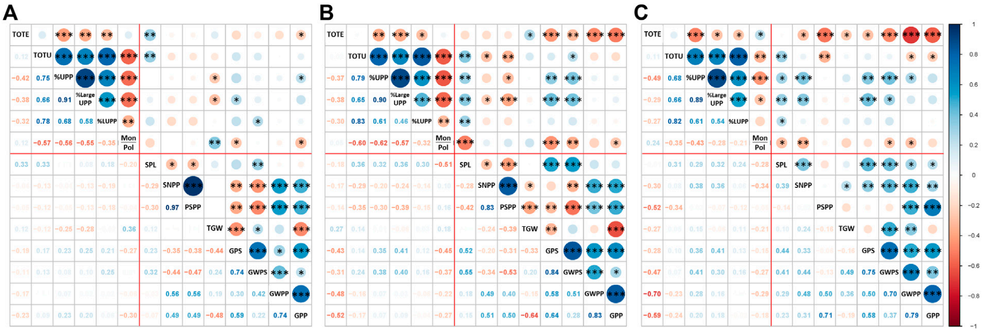

FIGURE 4
Correlation among protein composition traits and previously studied yield traits, total SDS-extractable proteins, total SDS-unextractable proteins, the percentage of SDS-unextractable polymeric protein in the total polymeric protein, the percentage of large SDS-unextractable polymeric protein in the total large polymeric protein, the percentage of large SDS-unextractable monomeric protein in the total large monomeric protein, the ratio of total monomeric proteins to the total polymeric proteins, spike length, spike number per plant, productive spikes per plant, 1000-grain weight, grains per spike, grain weight per spike, grain weight per plant, and grains per plant under **(A)** control **(C)**, **(B)** early drought, and **(C)** late drought stress. The significance level for Pearson's correlation coefficient was indicated by ***: $p < 0.001$, **: $p < 0.01$, and *: $p < 0.05$.

In the control group (C), two protein traits (TOTE and TOTU) correlated significantly and positively with the spike length (SPL); meanwhile, correlations between protein traits and grain yield traits were mainly lacking (Figure 4A). For the EDS and LDS plants, a higher number (than for C plants) of significant relationships were found among protein parameters and yield traits. Thus, several of the protein parameters (TOTU, %UPP, %LargeUPP, and %LUMP) correlated significantly and positively with SPL, but TOTE and Mon/Pol correlated significantly and negatively with grain yield components (Figures 4B, C).

3.4 Genotypes with the highest and lowest 10% of TOTE

The 10% genotypes with the highest and lowest TOTE showed values of 1.69–2.06 × 10^8 and 0.90–1.03 × 10^8 under C, 1.73–1.99 × 10^8 and 1.13–1.19 × 10^8 under EDS, and 3.27–3.94 × 10^8 and 1.33–1.63 × 10^8 under LDS, respectively (Table 1). The 10% genotypes with the highest TOTE were represented by wheat–rye introgression genotypes across three treatments, except for one modern and one old genotype appearing in LDS. In contrast, primarily modern and old genotypes were found among the 10% of the genotypes with the lowest TOTE under C and EDS, respectively.

A PCA comparing the effect of eight previously obtained yield components (SPL, SNPP, PSPP, TGW, GPS, GWPS, GWPP, and GPP) on the 10% genotypes with the highest and lowest TOTE clearly showed the impact of the yield components on the grain protein concentration (TOTE) for all treatments (Figure 5). High-yield components were related to low TOTE for both the C treatment (Figure 5A) and the EDS (Figure 5B) and LDS (Figure 5C). However, a few genotypes, 252 (3R), 253 (3R), and specifically, 258 (2R), were found with both high values on the yield components and high TOTE under the C treatment (Figure 5B).

3.5 Genotypes with the highest and lowest %UPP

The 10% genotypes with the highest and lowest %UPP showed values of 45.29%–57.79% and 12.24%–23.29% under C, 44.65%–55.29% and 15.05%–23.64% under EDS, and 45.01%–59.41% and 15.39%–22.53% under LDS, respectively (Table 2). The old genotypes were found to dominate the 10% genotypes with the highest %UPP while 1R and 1RS genotypes dominated the 10% genotypes with the lowest %UPP, in both cases, across all three treatments.

A PCA comparing the effect of eight yield components (SPL, SNPP, PSPP, TGW, GPS, GWPS, GWPP, and GPP) on the 10% genotypes with the highest and lowest %UPP showed a clear impact of spike characters (GPS—grains per spike, GWPS—grain weight per spike, and SPL—spike length) on %UPP, independent of treatment (Figures 6A–C). Thus, in principle, a higher protein polymerization is obtained with increased spike size.

Under LDS, additional yield components were found related to the 10% genotypes with the highest %UPP (Figure 6C). However, this relationship might be partly a result of the strong negative correlation between grain yield components (GWPS, GWPP, and GPP) and grain protein concentration (TOTE) and between grain protein concentration (TOTE) and gluten strength (%UPP) under LDS.

3.6 Genotypes with the highest stability and adaptability of TOTE and %UPP

The 10% most stable and adaptable genotypes in TOTE showed the harmonic mean of genotypic value of 1.91–2.12 × 10^8, the relative performance of genotypic value of 1.18–1.32, and the harmonic mean of relative performance of genotypic value of

TABLE 1 **10% genotypes with the highest and lowest grain TOTE under control, early drought stress, and late drought stress treatments.**

Treatment	Highest 10% genotype	Genetic background	TOTE (10^8)	Lowest 10% genotype	Genetic background	TOTE (10^8)
C	260	NA	2.06	225	1R	0.90
	253	3R	1.99	274	Modern	0.98
	266	3RS	1.98	196	Old	1.00
	252	3R	1.72	273	Modern	1.02
	258	2R	1.71	281	Modern	1.02
	263 (2)	1R+6R	1.69	271	2R	1.03
	262	5R	1.69	277	Modern	1.03
EDS	253	3R	1.99	207	Old	1.13
	252	3R	1.87	225	1R	1.13
	224	1R	1.83	204	Old	1.14
	221	1R	1.82	208	Old	1.17
	258	2R	1.81	212	1R	1.18
	263	5R	1.79	197	Old	1.18
	260	NA	1.73	223	1R	1.19
LDS	257	3R	3.94	265	3RS	1.33
	209	Old	3.82	273	Modern	1.34
	245	2R	3.58	268	NN	1.37
	277	Modern	3.42	281	Modern	1.56
	235	1RS	3.39	266	3RS	1.58
	250	3R	3.35	225	1R	1.59
	260	NA	3.27	270	2R	1.63

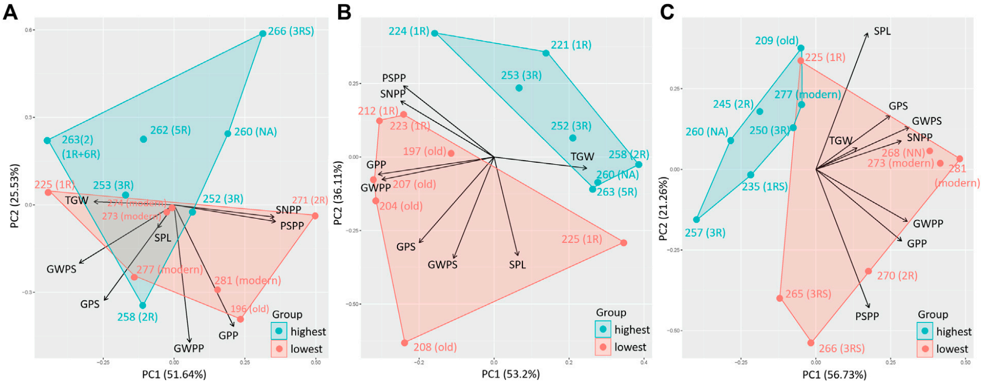

FIGURE 5
Principal component analysis for yield components, spike length, spike number per plant, productive spikes per plant, 1000-grain weight, grains per spike, grain weight per spike, grain weight per plant, and grains per plant of genotypes with the highest 10% (highest) and lowest 10% (lowest) TOTE under **(A)** control **(C)**, **(B)** early drought, and **(C)** late drought stress.

TABLE 2 10% genotypes with the highest and lowest %UPP under control, early drought stress, and late drought stress treatments.

Treatment	Highest 10% genotype	Genetic background	% UPP (%)	Lowest 10% genotype	Genetic background	% UPP (%)
C	225	1R	57.79	223	1R	12.24
	206	Old	49.54	221	1R	21.78
	205	Old	48.56	260	NA	22.00
	248	3R	46.68	267	1RS	22.60
	202	Old	46.27	198	Old	22.65
	207	Old	46.15	229	1RS	23.15
	200	Old	45.29	222	1R	23.29
EDS	225	1R	55.29	223	1R	15.05
	206	Old	50.74	198	Old	21.14
	212	1R	49.66	263 (2)	1R+6R	22.05
	205	Old	49.24	221	1R	22.81
	200	Old	46.25	267	1RS	23.11
	279	Modern	45.49	222	1R	23.50
	248	3R	44.65	224	1R	23.64
LDS	225	1R	59.41	257	3R	15.39
	206	Old	48.90	260	NA	18.44
	248	3R	46.39	220	1R	18.66
	204	Old	46.02	223	1R	19.06
	205	Old	45.84	198	Old	19.65
	281	Modern	45.62	235	1RS	21.19
	215	1R	45.01	245	2R	22.53

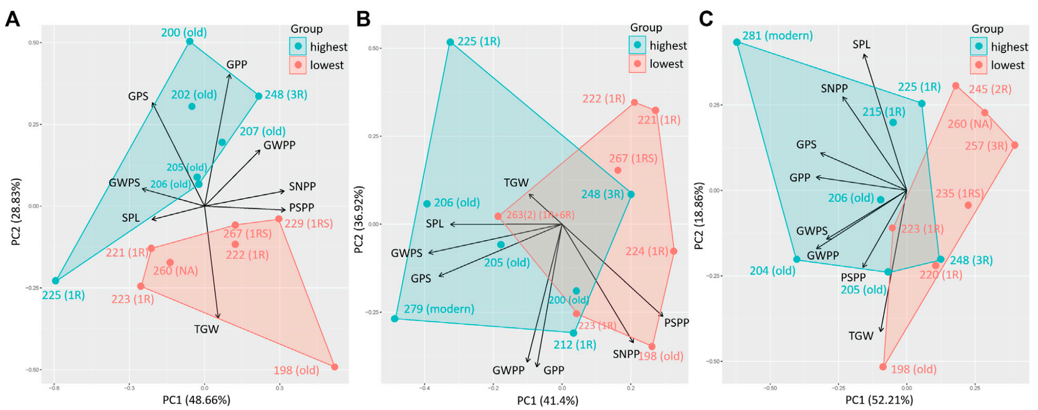

FIGURE 6
Principal component analysis for yield components, spike length, spike number per plant, productive spikes per plant, 1000-grain weight, grains per spike, grain weight per spike, grain weight per plant, and grains per plant of genotypes with the highest 10% (highest) and lowest 10% (lowest) %UPP under **(A)** control **(C)**, **(B)** early drought, and **(C)** late drought stress.

TABLE 3 10% genotypes with the highest, most stable, and most adaptable TOTE and %UPP based on BLUP indexes including the harmonic mean of genotypic values indicating stability, the relative performance of genotypic values indicating adaptability to drought stress, and the harmonic mean of the relative performance of genotypic values indicating stability and adaptability simultaneously.

	Highest 10% genotype	Genetic background	HMGV (TOTE: 10^8, %UPP: %)	RPGV	HMRPGV
TOTE	260	NA	2.12	1.32	1.31
	253	3R	2.12	1.30	1.29
	257	3R	1.92	1.27	1.24
	250	3R	1.91	1.23	1.22
	258	2R	1.94	1.20	1.20
	245	2R	1.84	1.21	1.19
	252	3R	1.91	1.18	1.16
%UPP	225	1R	56.86	1.58	1.58
	206	Old	49.33	1.37	1.37
	205	Old	47.51	1.32	1.32
	248	3R	45.61	1.27	1.27
	202	Old	43.92	1.22	1.22
	204	Old	43.57	1.21	1.21
	207	Old	43.44	1.21	1.21

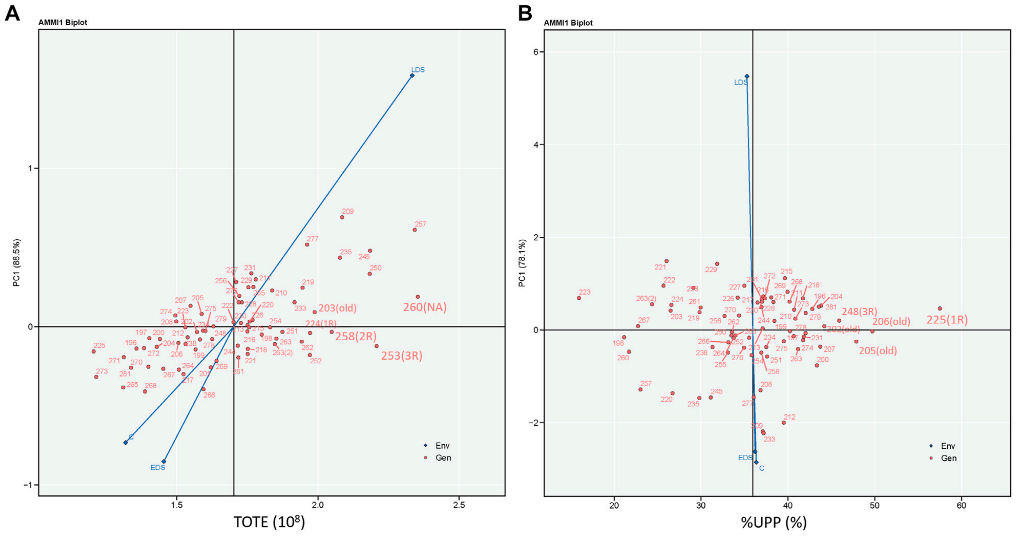

FIGURE 7
Additive main effects and multiplicative interaction biplots showing **(A)** TOTE (10^8) and **(B)** %UPP (%) versus the first principal component score of 73 genotypes and three growing conditions including control (abbreviated as C), early drought stress (EDS), and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) had relatively higher stability in TOTE and %UPP across three growing conditions.

1.16–1.31. Meanwhile, the 10% most stable and adaptable %UPP ranged from 43.44%–56.86% in HMGV, 1.21–1.58 in RPGV, and 1.21–1.58 in HMRPGV (Table 3).

Genotype 260, also identified as the highest in Table 1 under C, showed the most stable grain protein concentration (TOTE) performance simultaneously with the highest HMGV (2.12 × 10^8), RPGV (1.32), and HMRPGV (1.31). Genotype 225, also identified as the highest in Table 2 under C, EDS, and LDS, showed the most stable gluten strength (%UPP) simultaneously with the highest HMGV (56.86%), RPGV (1.58), and HMRPGV (1.58; Table 3). Genotypes with chromosome 3R (253, 257, 250, and 252) and old genotypes (206, 205, 202, 204, and 207) outnumbered other genotype groups among the 10% most stable TOTE and %UPP genotypes, respectively (Table 3).

The additive main effects and multiplicative interaction identified the strongest interaction force under LDS due to its longest vector, and C and EDS showed similar interaction forces with genotypes for both TOTE and %UPP (Figure 7). The highest and most stable TOTE was found in genotype 260 (NA), followed by 253 (3R), 258 (2R), 203 (old), and 224 (1R), with relatively high and stable values of TOTE (Figure 7A). Meanwhile, the highest and most stable %UPP was found in genotype 225 (1R), followed mainly by old genotypes 206 (old), 205 (old), 248 (3R), and 202 (old), with relatively high and stable values of %UPP (Figure 7B).

4 Discussion

The present study clearly showed the importance of the sources of wheat germplasm for the performance in terms of grain protein composition under drought stress. Genotypes containing 3R were well represented with high TOTE both during control conditions and at EDS and LDS. Thus, these genotypes were also found stable for TOTE over optimal and drought conditions at various stages of wheat development. A high %UPP was found in some of the old genotypes at control conditions and also at EDS and LDS, which resulted in the highest stability for some of the old genotypes across control and drought conditions. However, part of the variations in grain protein concentration (TOTE) was related to yield differences among the genotypes, and also, a correlation prevailed between gluten strength (%UPP) and some of the plant physiological parameters. Thus, when searching for genotypes with high stability across environments in quality traits, the relationship resulting in decreases in yield has to be taken into consideration. However, the 3R genotypes of the present study have previously been shown with a high yield at EDS, indicating that a reduction in yield is not the major explanation for the high grain protein concentration in these genotypes.

The significantly higher grain protein content, measured as TOTE, in 3R genotypes than in 1R, 1RS, 2R, modern, and old genotypes under C, indicated the presence of genes in 3R that contribute to high grain protein concentration. The low TOTE in modern wheat may be attributed to a high yield performance of these genotypes, with a high transfer of starch to the grains, thereby diluting the protein content. Unlike modern wheat, known to have high and stable yield performance as a result of yield-oriented breeding programs, the introgressed wheat lines have been developed attempting to introduce resistance genes from wild or distant relatives to wheat. Several successful transfers of rye genes have been reported, which contribute to disease resistance in adapted wheat cultivars (Friebe et al., 1996; Rahmatov, 2016; Crespo-Herrera et al., 2017). The chromosome 3R has been reported to increase stem rust resistance (Miller, 1984; Rahmatov, 2016), tolerance to early drought (Lan et al., 2022), and aluminum (Salvador-Moreno et al., 2018) in wheat. However, there is no report about the effect of 3R on breadmaking quality. In addition, studies on the contribution of 3R in breeding are fewer than those on other rye chromosome transfers, such as from 1R. The lack of studies on 3R may be attributed to its poor transmission rate (25.0%) compared to 1R (51.6%) and 2R (51.6%) while performing backcrossing (Li et al., 2018). In addition to contributing to high TOTE at control conditions, 3R was also found to contribute to stability in TOTE across drought environments, as demonstrated by the BLUP-based values of HMGV, RPGV, and HMRPGV. Thus, three wheat–rye introgression genotypes (252, 253, and 258) were found with both high TOTE and yield, as well as with high stability of TOTE across drought environments. Furthermore, the results of AMMI confirmed the high and stable TOTE of 253 and 258, and it also identified the strongest genotype × environment interaction in LDS. As our results demonstrated a significantly higher grain protein concentration with 3R than other rye chromosomes (1R, 1RS, and 2R) and genetic backgrounds (modern and old), and also stability across environments, without a reduction in yield, studies should be performed to identify the genes behind these characteristics and to further explore the effect of 3R on wheat grain quality.

The negative relationship between TOTE and yield traits identified in a correlation analysis was further confirmed by a PCA evaluating the impact of yield components on the highest and lowest TOTE. Across all three treatments, a high yield was mainly associated with the 10% genotypes with the lowest TOTE. A negative yield–protein relationship in wheat has been reported by a range of studies (Kibite and Evans, 1984; Simmonds, 1995; Feil, 1997; Cooper et al., 2001) and has long been viewed as the main difficulty in developing cultivars with both high protein content and yield (Blackman and Payne, 1987). Both grain yield and protein concentration of wheat have been reported as highly prone to genotype × environment interactions (Oury et al., 2003; Asseng and Turner, 2007). However, grain protein concentration and grain yield have been assigned as separate characters governed by different genes. Instead, this negative yield–protein correlation is attributed to the fact that grain yield is closely related to the starch content in the grain (Bhullar and Jenner, 1985; Hakim et al., 2012). Thus, a decrease in grain starch content results in a reduction in yield, and if not, protein accumulation is impacted in a higher grain protein concentration. In this study, three of the top 10% TOTE genotypes were found with high yield components, i.e., 252 (3R) with a relatively high number of spikes (SNPP and PSPP), 253 (3R) with large grain size (TGW), and 258 (2R) with both high grain number (GPS) and grain weight (GWPP). The fact that the yield components responsible for the high yield despite high TOTE varied among the three genotypes identifies different genetic-based potential breeding solutions for a combination of high yield and high protein concentration in the same genotype. Thus, the genetic backgrounds for these phenotypic characteristics require further investigation.

All genotypic and environmental characters impacting plant development, e.g., days to heading and days to anthesis, have, in previous studies, been shown to affect %UPP and, thereby, gluten strength and breadmaking quality (Malik, 2012; Johansson et al., 2013; Johansson et al., 2020). Thus, factors such as nitrogen regimes and temperature have been shown largely impacting protein polymerization and gluten strength (Johansson et al., 2013; Gagliardi et al., 2020). However, the drought conditions used in the present study have previously been shown not to significantly alter the days to heading or days to anthesis (Lan et al., 2022). These findings correspond well with the fact that no significant differences in %UPP were found among C and the drought treatments EDS and LDS used here. However, despite no general effect of drought on %UPP being noted, significant variations were found for genotype groups. Thus, 1R genotypes showed a significantly lower %UPP than old genotypes under C, and moreover, the %UPP of both 1R and 1RS was significantly lower than that of both modern and old genotypes under EDS, verifying 1R and 1RS, contributing to a decreased gluten strength, as has been described in previous studies (Graybosch et al., 1993; Fenn et al., 1994). The 1B chromosome, substituted to 1R, is known to contain genes for gluten proteins important for gluten strength, and the secalins that the 1R adds cannot contribute similar properties (Josephides et al., 1987; Graybosch et al., 1993). The significantly higher Mon/Pol found in 1RS than in 1R, 2R, and 3R, as well as modern and old genotypes in the present study, also indicates the issue with less polymerization of the proteins related to the substitution of gluten genes in 1B with secalin genes in 1R. This increased Mon/Pol from 1RS obtained across all three treatments further suggested that genes on the short arm of 1R played a major role in forming weak and sticky dough as these quality defects were independent of environments. Despite the negative effects on breadmaking quality brought by 1RS, it is still one of the most widely used sources of alien genes for wheat breeding due to several traits being improved with the successful introgression of 1RS, especially disease resistance, such as yellow rust (Yang et al., 2016), stem rust (Mago et al., 2002), leaf rust (Hsam et al., 2000), and powdery mildew (Mohler et al., 2001). Furthermore, 1RS was also reported to increase root length (Lan et al., 2022), drought tolerance (Ludlow and Muchow, 1990), and final yield (Ren et al., 2018).

Regarding genotypes with the highest and lowest 10% %UPP, old genotypes and 1R (including 1RS) dominated the highest and lowest %UPP ranking, respectively, which implied high gluten strength in old Swedish cultivars and further confirmed the poor breadmaking quality brought by 1R and 1RS, as discussed above. Among different yield components, GPS, GWPS, and SPL were found to relate to high %UPP across the three treatments, indicating the association between increased spike size and high gluten strength. Previous studies have reported a positive correlation between grain weight and gluten strength measured by mixograph peak height (Tahir et al., 2006) and a negative correlation between spike density and gluten strength indicated by sodium dodecyl sulfate (SDS) sedimentation volume (Hailu and Merker, 2008). However, the eventual relationship between spike size and gluten strength, as reported here, needs to be further elaborated on, and genes for its determination need to be further determined. The fact that protein composition in the grains differs along the wheat spike (Johansson et al., 2013) may contribute to the results obtained. In addition to showing a high %UPP, old Swedish cultivars also showed a stable %UPP over control and drought stress conditions of different times during plant development. All the five old genotypes (202, 204, 205, 206, and 207) identified as among the 10% of genotypes with the highest %UPP and also showed high stability by HMGV, RPGV, and HMRPGV, and the high-and-stable %UPP attribute of 202, 205, and 206 was confirmed by AMMI. In general, high gluten strength has not been attributed to old wheat genotypes in previous studies (Fois et al., 2011; De Santis et al., 2017), which correspond to the results of the present study, where old genotypes all over do not show significantly higher %UPP values than modern wheat, and the %LargeUPP is even significantly lower than in modern wheat for some treatments. Thus, from the present study, it is shown that some specific old genotypes were identified with high and stable gluten strength over control and drought environments. The genetic background for this performance needs to be further elaborated on.

Grain yield and quality are the two factors determining the sufficiency and usefulness of wheat, respectively. Unfortunately, both are under threat of drought stress with the increasing global temperature (Allen et al., 2019). The extensive daily consumption of different types of wheat products such as bread, pastries, noodles, biscuits, and porridge amplifies the challenges of climate change on the end-use quality (Johansson et al., 2020; Johansson et al., 2023), which urged the need to improve wheat processing quality, especially from genetic aspects. Using molecular markers, several studies have reported the identification of responsible QTLs for grain protein concentration in common bread wheat (Prasad et al., 2003; Leonova et al., 2022), durum wheat (Blanco et al., 2006), and synthetic hexaploid wheat with emmer background (Kunert et al., 2007). By manipulating the expression of storage protein genes, satisfactory protein content was found to be retained while increasing the yield by altering field nitrogen supply and plant density (Zheng et al., 2022). However, due to the genetic complexity of wheat grain quality traits, the utilization of specific genes (especially transferred from alien species or wild relatives) for improving protein traits is difficult (Kulwal et al., 2005; Balyan et al., 2013). Therefore, valuable genetic resources need to be tested more widely in future studies to achieve successful quality breeding.

5 Conclusion

Global climate change is threatening food security in two aspects, i.e., quantity and quality. Improvements in the stability of the breadmaking quality of wheat are urgently needed against increasing drought events. The baking quality is determined by its protein composition parameters, especially the protein concentration and gluten strength, which are known to be affected by genotype × environment interactions. Therefore, in search of genotypic resources and candidate genes contributing to high and stable quality, this study investigated the impact of drought on the protein composition of wheat materials with a wide genetic background. Due to its highest total extractable proteins among genotype groups, chromosome 3R demonstrated a promising effect in increasing protein concentration that has been reduced in modern breeding lines. Furthermore, the simultaneous high performance in protein content and yield found in 252(3R), 253(3R), and 258 (2R) proposed possibilities of using wheat-alien introgression lines as genetic resources to achieve high protein quality without losing yield. Our stability analysis results also supported the contribution of 3R to high and stable protein content across varying environments. Differently, some of the old Swedish cultivars were found to be a potential genetic resource for high and stable gluten strength. Thus, this study identifies 3R and some specific old Swedish cultivars as the two major sources for genes related to protein concentration and gluten strength. Breeding targets need to be adjusted accordingly to achieve wheat lines with stable and high baking quality under increasing drought conditions.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

EJ, RK, AC, and YL contributed to conception and design of the study. YL collected the raw data. YL performed the statistical analysis. YL wrote the first draft of the manuscript. EJ, RK, and AC provided supervision on this work. EJ contributed to project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frfst.2023.1163412/full#supplementary-material

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III

PLOS ONE

RESEARCH ARTICLE

Chasing high and stable wheat grain mineral content: Mining diverse spring genotypes under induced drought stress

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Abstract

Climate change-induced drought has an effect on the nutritional quality of wheat. Here, the impact of drought at different plant stages on mineral content in mature wheat was evaluated in 30 spring-wheat lines of diverse backgrounds (modern, old and wheat-rye-introgressions). Genotypes with rye chromosome 3R introgression showed a high accumulation of several important minerals, including Zn and Fe, and these also showed stability across drought conditions. High Se content was found in genotypes with chromosome 1R. Old cultivars (K, Mg, Na, P and S) and 2R introgression lines (Fe, Ca, Mn, Mg and Na) demonstrated high mineral yield at early and late drought, respectively. Based on the low nutritional value often reported for modern wheat and negative climate effects on the stability of mineral content and yield, genes conferring high Zn/Fe, Se, and stable mineral yield under drought at various plant stages should be explicitly explored among 3R, 1R, old and 2R genotypes, respectively.

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Introduction

Global food security is highly challenged both in terms of adequacy and nutritional value as a result of an increasing world population, ongoing climate change and unstable human conditions due to migration, poverty and conflicts (wars). This has led to a sharp increase in the global prevalence of undernourishment from 7.9% in 2019 to 9.3% in 2020 and remained at a high level (9.2%) until 2022 [1]. Intake of mineral nutrients is essential in this context as they contribute to the absorption and function of vitamins by the human body [2]. Among the minerals, zinc (Zn) and iron (Fe) have been described as the ones of utmost importance to human health. Zinc deficiency has been recognized as a threat to public health as it links broadly to weakened immunity, liver disease and diarrheal problems [3,4], while the anemia caused by Fe deficiency is the most common health issue worldwide [5,6]. Additionally, selenium (Se) plays a critical role for the human immune function, brain function, male fertility and type-2 diabetes risks [7,8]. Furthermore, calcium (Ca) is widely involved in life processes in cells and bone health [9,10]; copper (Cu) is tightly intertwined with the metabolism of other minerals, i.e. Cu deficiency results in Fe deficiency [11]; magnesium (Mg) has been reported to have a function of activating vitamin D in the human body [12]; manganese (Mn) is essential for the activation of metalloenzymes [13], and potassium (K) and sodium (Na) actively play a role in neurologic and muscular systems, where the Na-K flux on membranes is known to drive nerve impulses by

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changing the electrical potential [14]; phosphorus (P) intake at high levels is associated with an increased risk of cardiovascular disease [15,16]; Dietary sulfur (S) is supplied to humans from various sources e.g. vegetables (allium and brassica species), legume crops and animal-based products. It plays a role in protein synthesis in the form of the two primary S-containing amino acids methionine and cysteine [17,18] and is stored as a key metabolite glutathione [19].

In plants, mineral elements are mainly absorbed as ions from the soil, and their content and composition play a key role in plant growth and reproduction [20]. The amount of Zn is extremely important for the enzyme activity of the plants, as it is present in six important plant enzyme classes i.e., oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases [21]. Iron is important for plants as it accumulates in chloroplasts in the green leaves, based on its indispensable role in photosynthetic activities [22,23]. Differently from Zn and Fe, Se is not an essential element for plants although an appropriate amount of Se contributes to plant growth and stress tolerance [24–26]. In addition, other mineral elements e.g. Ca, Cu, Mg, Mn, K, Na, P and S, are also required by the plant to sustain most physiological processes such as photosynthesis, enzyme activation, protein synthesis and pollen formation [23,27]. Thus, mineral accumulation is closely related to both the healthy growth and nutritional value of a crop.

Drought, the major abiotic yield-limiting factor [28], is also known to impact the process of nutrient uptake of a plant due to the drought-induced physiochemical (nutrient mobility and absorbance) changes in soils [29]. Water deficit conditions in the soil are known to negatively affect the mineral uptake in the plants by i) impaired uptake power in the root due to inhibited activity and ii) limited ion diffusion rate due to the low moisture level. However, drought conditions are also known to reduce the content of mineral elements in the plant as transpiration rate and membrane permeability are restricted [30,31]. By the predicted increase in drought events due to climate change, an increased understanding of the effects of drought on mineral content in plants is urgently needed to achieve a high and stable nutritional content in crops.

Wheat (*Triticum aestivum*), one of the three major cereal crops, is feeding the world population with a share of approximately 20% of the calories and proteins [32]. Because of the high daily consumption, wheat products are a crucial source of nutrition for humans. However, similarly to other crops wheat is facing an increasing number of drought spells because of climate change [33]. The timing of the drought spells contributes with different effects to the wheat, e.g. early drought resulted in the inhibition of morphologic traits while late drought restricted the yield [34]. The mineral concentration of the wheat grain is known to be determined by genetics, the environment and their interactions, although a general decrease in the mineral nutritional value of wheat grain has been reported as a result of breeding selections [35]. Drought during field conditions has been found to contribute to a significant increase in grain Zn concentration [36].

This study aimed to deepen the understanding of the impacts of early and late drought stress on wheat grain mineral composition (11 mineral elements). Combined with previously obtained grain yield data, the amount of each element was calculated to identify the single-plant-based nutritional value of wheat from different genetic backgrounds. Another aim was to identify genetic resources of high and stable nutritional value in terms of mineral amount for breeding programs using a wide array of wheat materials.

Materials and methods

Plant materials

A total of 30 spring wheat (*Triticum aestivum L.*) genotypes including modern (n = 5), old (n = 5), introgression wheat with rye chromosome 1R (n = 5), 1RS (n = 5), 2R (n = 5) and 3R (n = 5), selected from a previous investigation [34] were used in this study (S1 Table).

Growing conditions and drought treatments

Similarly as has been described previously [34], pPlants were grown under controlled climatic conditions from April to September, 2020 in the Biotron at the Swedish University of Agricultural Sciences in Alnarp, Sweden, using natural light and hourly-regulated temperature and humidity derived from the average climate data of Malmö, Sweden during the period of 2010–2019 (Swedish Meteorological and Hydrological Institute, SMHI). This experiment used 2.5 L pots filled with soil (product name: Exklusiv Blom och Plantjord 50 liter; article number: 1640; pH: 5.5–6.5) containing 50% of low humified peat, 33% of highly humified peat, 7% of gravel, 5% of leca balls (2–6 mm), 5% of clay with silicon provided by Emmaljunga Torvmull AB (https://www.emmaljungatorvmull.se/), Sweden. The total set of 30 genotypes was subjected to each of the three growing conditions i.e. one control and two drought treatments (EDS: early drought stress; LDS: late drought stress) with three biological replicates used within each condition. Plants grown under control were watered every second day until spike maturity. Both drought treatments were applied in the form of water-withholding, with EDS starting 30 days after sowing and lasting 4 weeks, and LDS starting 60 days after sowing and lasting 2 weeks.

Sample preparation

From each growing condition, three biological replicates of each genotype were sampled, resulting in a total of 270 samples. All grain samples were oven-dried at 40˚C for 24 h and then milled for 30 s into flour (mixer mill 400 MM, RETSCH). For digestion, 150 mg of each flour sample was mixed with 3 ml of nitric acid (69–70%, J.T.Baker-instra analyzed) and then the samples were subjected to autoclave (GETINGE, Sweden) conditions; 121˚C, 200 Kpa for 30 min. Thereafter, the digested samples were cooled down to room temperature, and then 27 ml of Milli-Q water was added to dilute each solution 10 times. Finally, a total of 10 ml of the solution of each sample was collected and used for mineral analysis.

Mineral determination and mineral yield calculation

Concentrations of Zn, Se and Mn were determined by inductively coupled plasma mass spectrometry (ICP-MS, Aurora Elite, Bruker, U.S.) while concentrations of Ca, Cu, Fe, K, Mg, Na, P and S were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8300, Perkin-Elmer, U.S.). To describe the amounts of minerals provided by the grains of a plant (mg/plant), the single-plant-based yield of each mineral was calculated by multiplying mineral concentration and grain yield (grain weight per plant). Standards used in the analysis were atomic spectrometry standards from Perkin-Elmer, SPEX, AccuStandard and Merck. Calibration of the ICP-OES instrument was done by using a mixed multicomponent standard at three concentrations within the factor of 50 and calibration was maintained with independent standards. The detection limit used was three times the standard deviation based on multiple determination of the blanks treated as the sample, were blanks were treated identically and together with the samples. All the mineral concentration, grain yield and mineral yield data can be found in S2 Table.

Data analysis

All statistical analyses were done in RStudio [37]. A two-way analysis of variance (ANOVA) was performed to detect variations between treatments and among genotypes. The pairwise comparisons (LSD post-hoc test) between genotype groups (modern, old, 1R, 1RS, 2R and 3R) and between treatments (C, EDS and LDS) were performed using the R package 'agricolae'. The linear regression presented in scatter plots were computed and visualized using R package 'ggplot2' and 'ggpmisc'. Principal component analysis (PCA) was computed using R packages 'ggfortify' and 'rgl'. The additive main effects and multiplicative interaction (AMMI) was performed using the R package 'metan' to identify genotypes with high and stable nutritional value.

Results

Genotypic variations in minerals in relation to drought stresses

ANOVA showed a highly significant effect of both genotype and drought treatment on the mineral grain concentration and yield of most of the 11 mineral elements (Zn, Fe, Se, Ca, Cu, K, Mn, Mg, Na, P and S) evaluated (S3 Table).

The PCA clearly divided the genotypes based on the three treatments (control, EDS and LDS) along the first principal component (PC1) axis, accounting for 50.1% and 75.0% of the variation for mineral concentration (Fig 1A) and mineral yield (Fig 1B), respectively. However, the mineral concentration largely overlapped under control and EDS, indicating a lack of impact from EDS on grain mineral concentration. For grain mineral concentration, the samples grown under LDS, generally showed more positive PC1 values than samples grown under control and EDS. The concentrations of all minerals, with the exception of Mn, were also located with positive PC1 values, indicating a positive correlation between mineral concentration and LDS treatment (Fig 1A). Also for mineral yield, LDS samples were clearly differentiated along PC1 with more positive values than for control and EDS samples (Fig 1B). However, the mineral yield of the different minerals was in this case clustered with negative PC1 values, indicating a negative correlation between mineral yield and LDS treatment (Fig 1B).

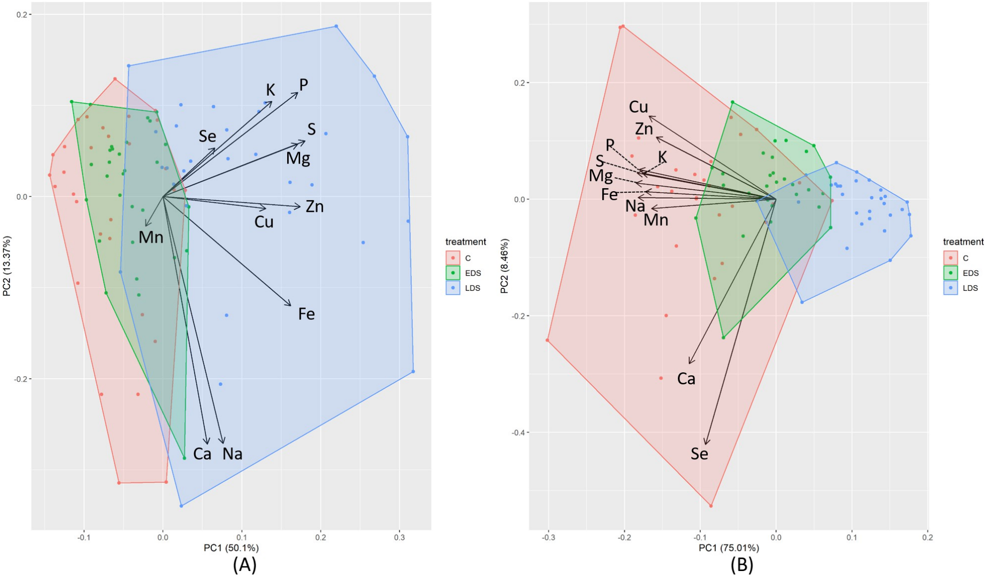

Fig 1. Biplots of principal component analysis (PCA) for the (A) grain concentration and (B) mineral yield of Zn, Fe, Se, Ca, Cu, K, Mn, Mg, Na, P and S of genotypes studied under control (C), early drought stress (EDS) and late drought stress (LDS) conditions.

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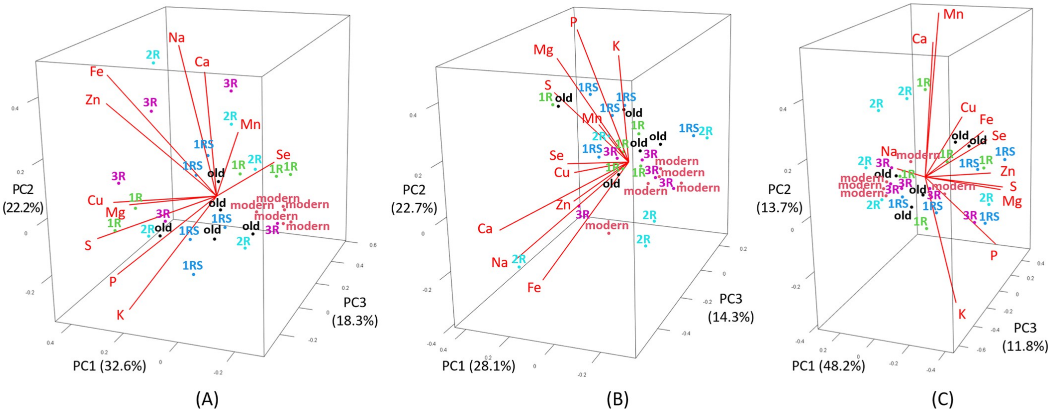

Fig 2. Principal component analysis (PCA) for grain concentrations of Zn, Fe, Se, Ca, Cu, K, Mn, Mg, Na, P and S in different genotype groups (modern = approved cultivars and breeding lines received from the breeding company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R) under (A) control, (B) early drought stress and (C) late drought stress.

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Relationships between mineral concentration/yield and genotype groups

The drought treatments affected the genotypes of various groups differently. Basically, all modern genotypes were found consistently with lower grain mineral concentrations than the other genotype groups across all the three treatments applied (control, EDS and LDS; Fig 2), indicating a relatively poor grain mineral nutrition of modern wheat compared with the other genotype groups. Differently, some old genotypes were found with high grain concentrations of Fe and Se, especially under LDS (Fig 2C). Furthermore, 1R genotypes showed a high grain concentration of Se and Mn under control (Fig 2A) while 1RS genotypes showed a high grain concentration of P, K and Mg under EDS (Fig 2B) and of Zn, Fe, Se, Mg, P and S under LDS (Fig 2C).

For mineral yield, modern genotypes generally showed the lowest values as compared to the other genotype groups (Fig 3A), but some modern genotypes were found with a high yield of some minerals under EDS (Cu, Fe; Fig 3B) and LDS (K; Fig 3C), which might be the result of a high grain yield of modern genotypes. Furthermore, some old genotypes showed high mineral yield for K, Mn, Mg, Na, P and S under EDS (Fig 3B). Also, 1R (Se and Mn) and 3R (Zn, Fe, Cu, K, Mg, P and S) genotypes were found with high mineral yield for different minerals under control (Fig 3A), while 2R genotypes were found with high mineral yield for Fe, Ca, Cu, Mn, Mg, Na, P and S under LDS (Fig 3C).

Grain concentration and mineral yield of Zn and Fe

Both grain Zn and Fe concentration and mineral yield of these components varied based on drought stress at different development stages (EDS and LDS) but also based on genotype groups (modern, old, 1R, 1RS, 2R and 3R). The Zn grain concentration was generally higher under LDS than under control for most of the genotype groups, with the exception of 2R genotypes (Fig 4A). Similarly, most genotype groups showed a higher Fe concentration under LDS than under control, with the exception of 2R and 3R, while only the modern genotype group displayed an increase in Fe concentration under EDS (Fig 4B).

A significantly higher Zn concentration was found for 3R genotypes (21.25 mg/kg) than most other genotype groups except the 2R genotypes under control while no difference was

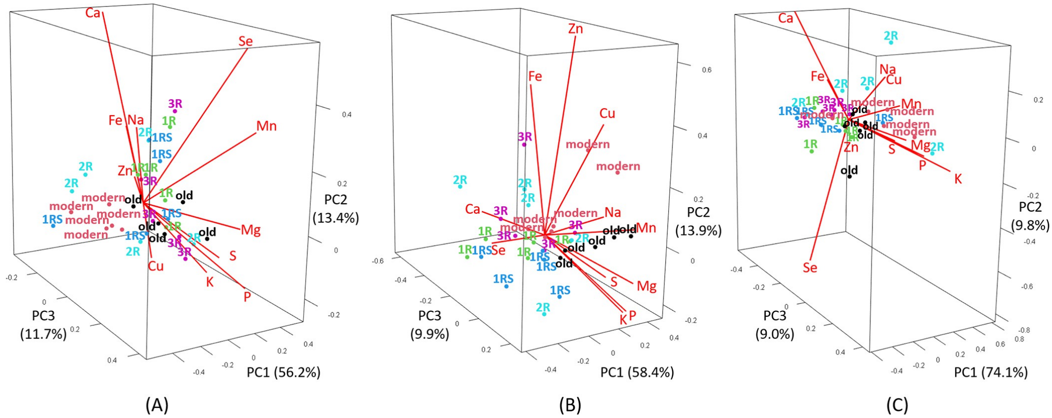

Fig 3. Principal component analysis (PCA) for mineral yield of Zn, Fe, Se, Ca, Cu, K, Mn, Mg, Na, P and S in different genotype groups (modern = approved cultivars and breeding lines received from the breeding company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R) under (A) control, (B) early drought stress and (C) late drought stress.

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found between genotype groups under EDS and LDS (Fig 4A). Both 2R (21.00 mg/kg) and 3R (21.81 mg/kg) genotypes showed a higher Fe concentration than modern genotypes (12.54 mg/kg) under control. Furthermore, a higher Fe concentration was found in 2R lines as compared to 1RS lines under EDS and in 1RS lines as compared to modern genotypes under LDS (Fig 4B).

No significant change in Zn yield by EDS and LDS treatments as compared to control was obtained for modern and old genotypes (Fig 4C). The Fe yield was significantly increased by EDS in modern genotypes, while LDS resulted in a decrease in Fe yield for both modern and old genotypes (Fig 4D). A decrease in both Zn and Fe yield under EDS as compared to control was obtained for 1R and 3R genotypes, while LDS reduced both Zn and Fe yield for all introgression lines (1R, 1RS, 2R and 3R; Fig 4C and 4D).

Significantly the highest Zn (0.11 mg/plant) and Fe (0.12 mg/plant) yield under control was found for the 3R genotypes (Fig 4C and 4D). Low Zn and Fe yield were obtained from 1R and 1RS lines under both EDS and LDS, while also 3R lines resulted in a low Zn and Fe yield under LDS (Fig 4C and 4D).

Genotypes with high concentrations and yield of Zn and Fe

A clear positive relationship between Zn and Fe was found for both grain concentration (Fig 5A) and yield (Fig 5D) for the evaluated genotypes under control ($R^2 = 0.63$ and 0.77, respectively). This positive relationship was decreased ($R^2 = 0.43$ and 0.35, respectively) under EDS (Fig 5B and 5E), and increased ($R^2 = 0.77$ and 0.81, respectively) under LDS (Fig 5C and 5F). Corresponding to the PCA results (Figs 2A and 3A), all the modern genotypes were located at the bottom left corner of the plots under control (Fig 5A and 5D), indicating a simultaneously low Zn and Fe in modern genotypes at non-drought conditions.

Two 3R genotypes (250 and 251) and one 2R genotype (258) with both high Zn and Fe grain concentrations (Fig 5A and S1A and S1D Fig), and three 3R genotypes (250, 251 and 256) with high Zn and Fe yield (Fig 5D) were identified under control. The genotypes 258 (2R) and 250 (3R; Fig 5B and S1B and S1E Fig), and the genotypes 250 (3R) and 279 (modern; Fig 5E) displayed high Zn and Fe grain concentration and mineral yield, respectively, under EDS

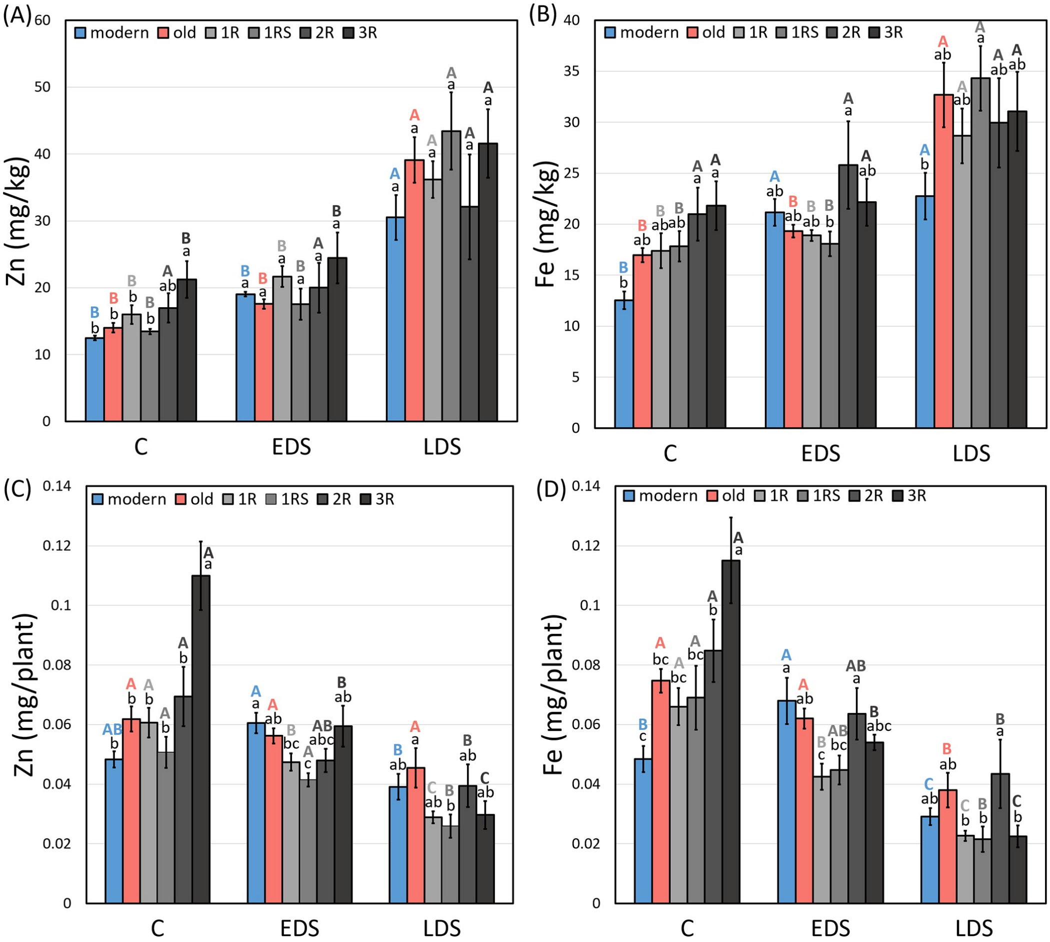

Fig 4. The mean grain concentration of (A) Zn and (B) Fe, and mineral yield of (C) Zn and (D) Fe of each genotype group under control (abbreviated as C), early drought (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same lowercase letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

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conditions. Furthermore, the genotypes 235 (1RS), 245 (2R) and 250 (3R; Fig 5C and S1C and S1F Fig) and the genotypes 207 (old) and 270 (2R; Fig 5F) showed high Zn and Fe grain concentration and mineral yield, respectively, under LDS.

Stability of the concentration and yield of Zn and Fe

The additive main effects and multiplicative interaction (AMMI) suggested a similar genotype × environment (treatments) interaction pattern between Zn and Fe grain

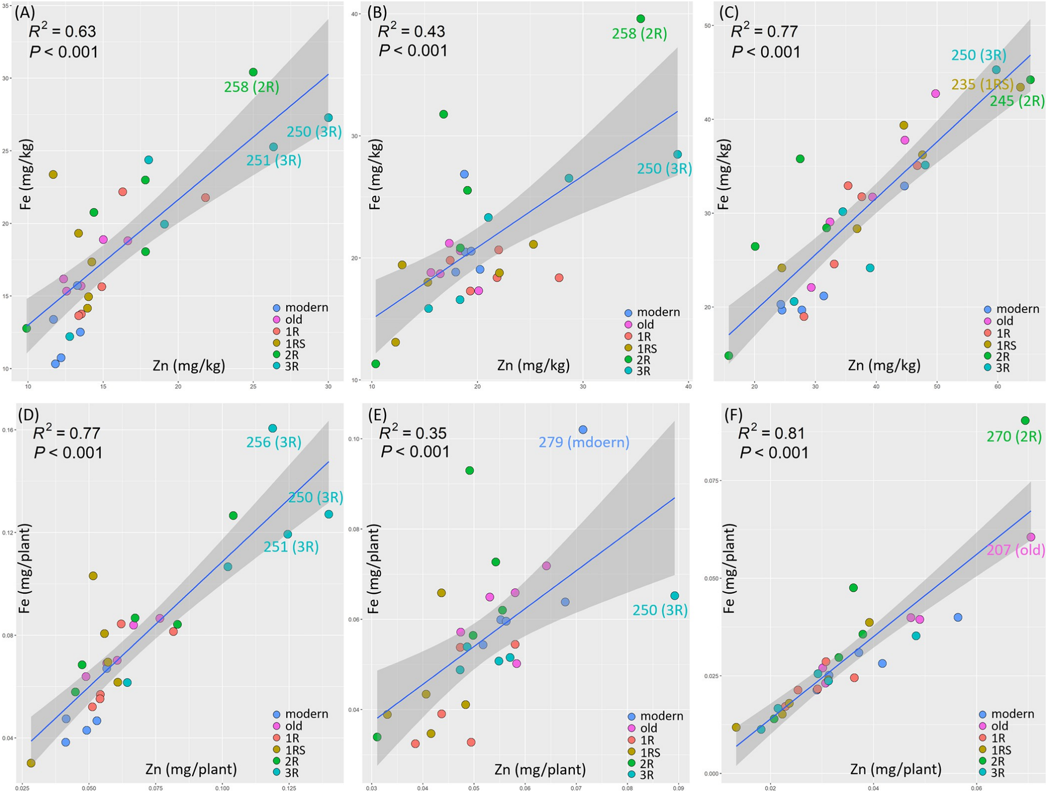

Fig 5. Linear regression (R^2 = the coefficient of determination) of Zn and Fe grain concentration and mineral yield in genotypes under (A and D) control (abbreviated as C), (B and E) early drought stress (EDS) and (C and F) late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R.

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concentration (Fig 6A and 6B), as well as between Zn and Fe yield (Fig 6C and 6D). For Zn and Fe concentrations, LDS showed the strongest interaction effect resulting in above-average concentrations while both control and EDS showed the interaction force resulting in below-average concentrations. Among the above-average genotypes, 250 (3R) and 251 (3R) were identified as high-and-stable genotypes for both Zn and Fe concentrations (Fig 6A and 6B). For Zn and Fe yield, the strongest interaction force was identified under control which resulted in above-average values, while LDS resulted in below-average values (Fig 6). Genotypes 250 (3R) and 251 (3R) showed high and stable Zn yield (Fig 6C) while genotypes 250 (3R), 251 (3R), 256 (3R), 258 (2R), 270 (2R) and 271 (2R) showed high and stable Fe yield (Fig 6D).

Concentration and yield of Se

Basically, no effect of drought stress was found on Se concentration (Fig 7A) while Se yield was decreased in 2R lines by EDS and in all introgression lines (1R, 1RS, 2R and 3R) by LDS as compared to control (Fig 7B). High grain concentration of Se was found in 1R genotypes at control, EDS and LDS conditions (Fig 7A).

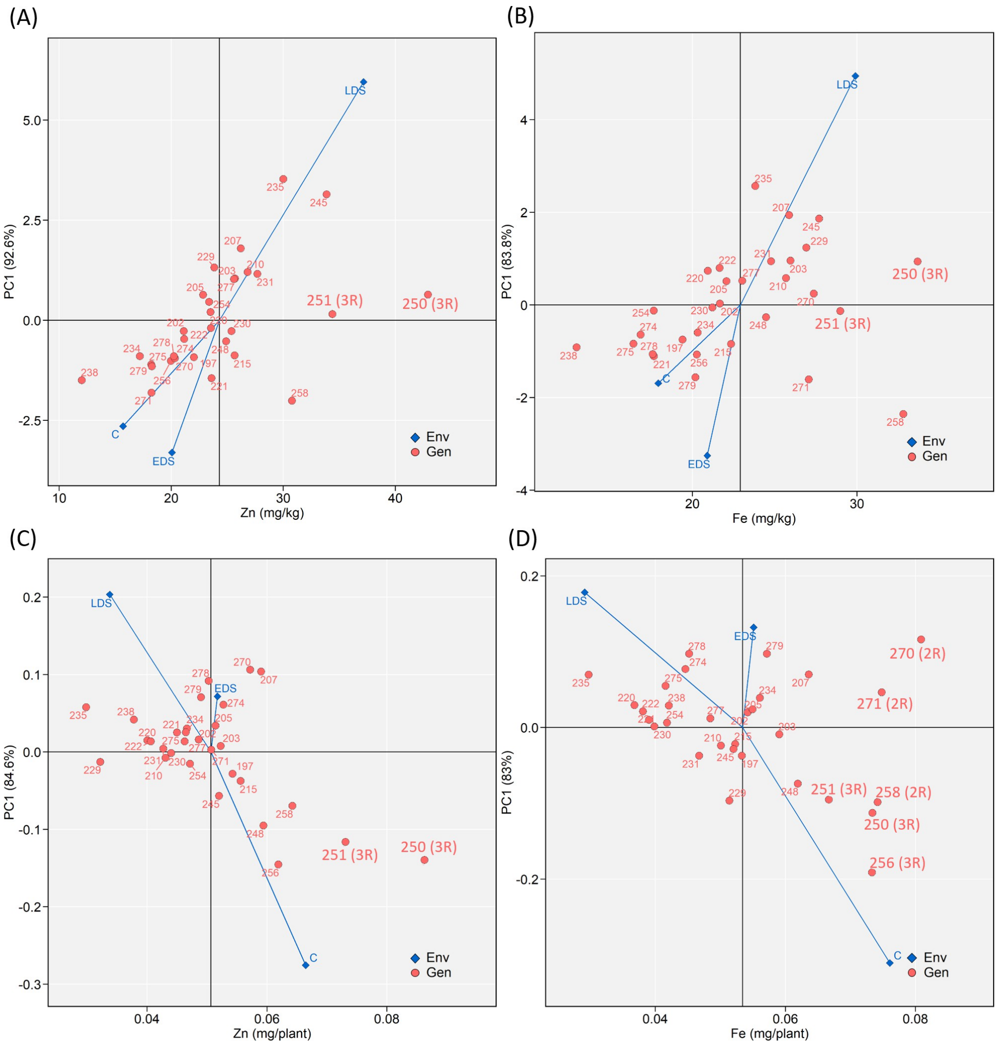

Fig 6. Additive main effects and multiplicative interaction (AMMI) biplots showing (A) Zn concentration, (B) Fe concentration, (C) Zn yield and (D) Fe yield versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) are those showing relatively higher stability across the three growing conditions. The vertical line in each figure indicates the average Zn and Fe grain concentration and mineral yield of the 30 genotypes.

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Stability of the concentration and yield of Se

The strongest genotype × environment interaction forces were identified under LDS and control for Se concentration and Se yield, respectively, and both resulted in above-average values (Fig 8). A more scattered distribution of the above-average genotypes was found for Se

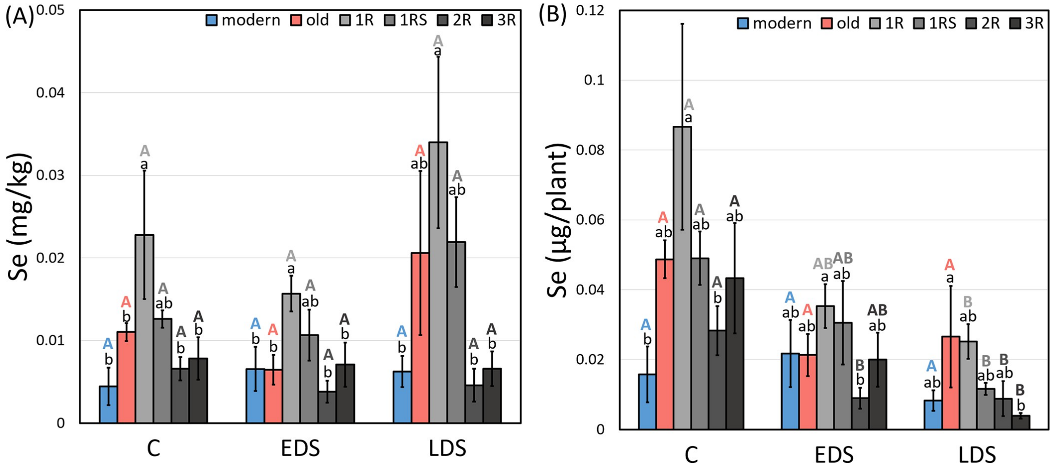

Fig 7. The mean (A) Se grain concentration and (B) Se mineral yield of each genotype group under control (abbreviated as C), early drought (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same lower letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

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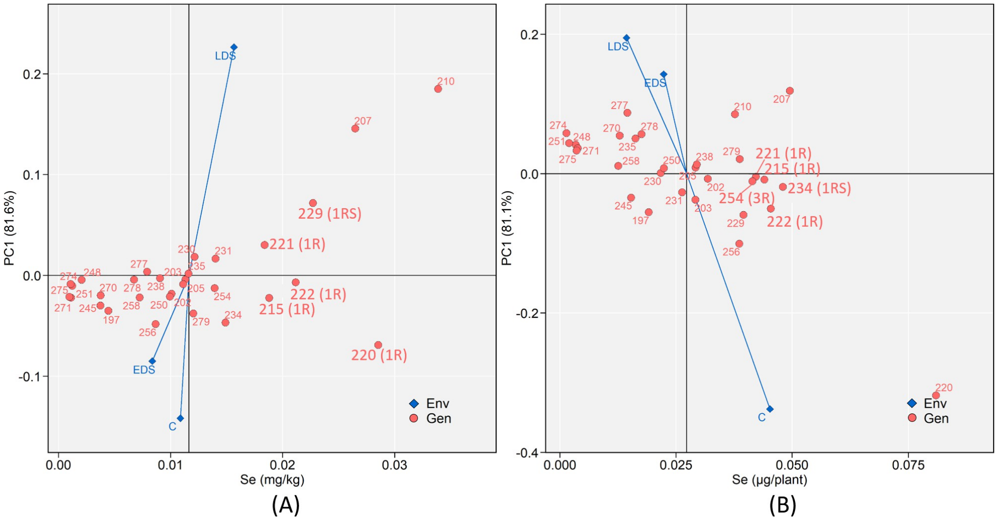

Fig 8. Additive main effects and multiplicative interaction (AMMI) biplots showing (A) Se grain concentration and (B) Se mineral yield versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) are those showing relatively higher stability across the three growing conditions. The vertical line in each figure indicates the average Se grain concentration and mineral yield of the 30 genotypes.

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concentration compared to the below-average genotypes (Fig 8A), indicating a clear dispersive effect from LDS. The genotypes 215 (1R), 220 (1R), 221 (1R), 222 (1R) and 229 (1RS) were identified as the high-and-stable genotypes for Se concentration (Fig 8A) while 215 (1R), 221 (1R), 222 (1R), 234 (1RS) and 254 (3R) were identified as the high-and-stable genotypes for Se yield (Fig 8B).

Concentration of Ca, Cu, K, Mn, Mg, Na, P and S

No effect of EDS or LDS was found on Ca concentration. 1RS genotypes showed a significantly higher Ca concentration than modern and old genotypes under control while 1RS genotypes maintained a higher Ca concentration than modern genotypes under LDS (Fig 9A).

An increase in Cu concentration was noted for 1R genotypes under EDS as compared to control, while LDS resulted in increases in Cu concentrations for modern, old and 1R genotypes. The 3R genotypes showed a higher Cu concentration than 1R, modern and old genotypes under control while no difference was found between genotypes groups under EDS and LDS (Fig 9B).

No effect from EDS was found on K concentration while significant increases were noted for modern, 1R, 1RS and 3R genotypes under LDS. Old genotypes showed a significantly higher K concentration than 2R genotypes under control while under EDS, old genotypes showed a higher value than 1R, 2R and 3R genotypes, and 1RS showed a higher value than 2R genotypes. No variation was found between genotype groups under LDS (Fig 9C).

Increases in Mn concentration were found for modern and 3R genotypes under EDS as compared to control while no effect of LDS was observed. The 1R genotypes showed high Mn concentration under control (higher than modern, 1RS, 2R and 3R) and EDS (higher than modern, old, 1RS and 3R) while under LDS, 1R genotypes only showed a significantly higher Mn concentration than 1RS and 3R genotypes (Fig 9D).

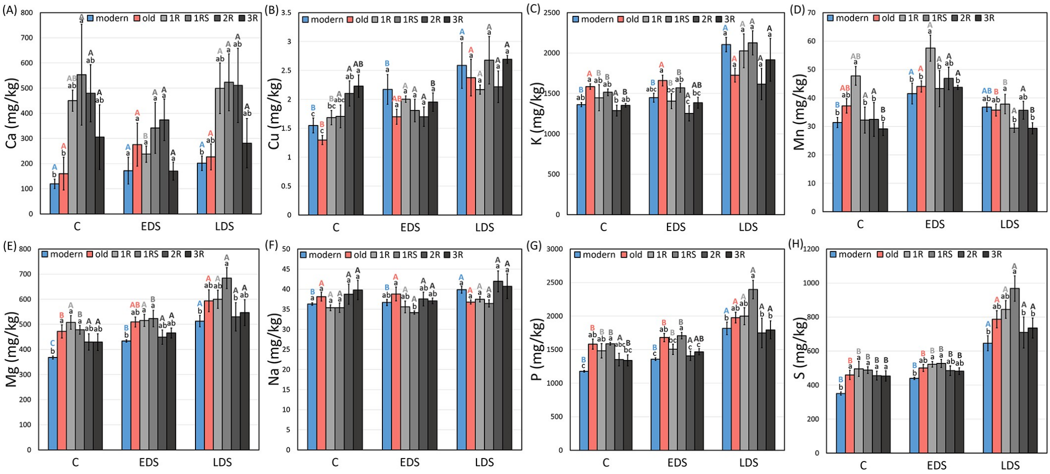

Fig 9. The mean (A) Ca, (B) Cu, (C) K, (D) Mn, (E) Mg, (F) Na, (G) P, (H) S concentration of each genotype group under control (C), early drought (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same lower letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

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A high Mg concentration was found for modern genotypes under control while under LDS, increases were noted in modern, old and 1RS genotypes. Modern genotypes showed a generally low Mg concentration under control (lower than old, 1R and 1RS) and EDS (lower than 1R and 1RS) while 1RS genotypes showed high concentrations (higher than modern and 2R) under LDS (Fig 9E).

The drought effect on Na concentration was only found in modern genotypes under LDS. The only difference between genotype groups was noted under EDS where old genotypes showed a higher Na concentration than 1RS genotypes (Fig 9F).

No effect from EDS was found on P concentration while under LDS, significant increases were noted for modern, old, 1R, 1RS and 3R genotypes. Under control, modern genotypes showed a lower P concentration than old, 1R and 1RS genotypes, and 1RS genotypes showed a higher value than 3R genotypes. Under EDS, 1RS genotypes showed a higher P concentration than modern, 1R, 2R and 3R genotypes, and old genotypes showed a higher value than modern, 2R and 3R genotypes. 1RS genotypes maintained a higher P concentration than modern, 2R and 3R genotypes under LDS (Fig 9G).

No effect from EDS was found on S concentration while all the genotype groups showed an increase under LDS. Significantly the lowest S concentration was found for modern genotypes under control. 1R and 1RS genotypes showed a higher S concentration than modern genotypes under EDS while 1RS showed a higher value than modern, 2R and 3R genotypes under LDS (Fig 9H).

Yield of Ca, Cu, K, Mn, Mg, Na, P and S

A decrease in Ca yield was found for 1R genotypes under EDS while 1R and 1RS genotypes showed a decrease under LDS. 1RS genotypes showed a significantly higher Ca yield than modern genotypes under control while 2R genotypes showed a higher value than modern, old, 1RS and 3R genotypes under LDS (Fig 10A).

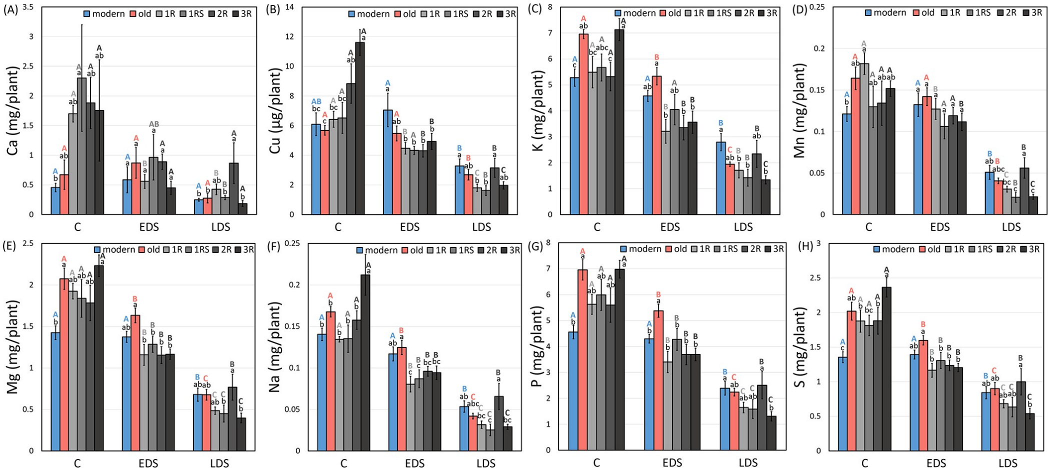

Fig 10. The mean (A) Ca, (B) Cu, (C) K, (D) Mn, (E) Mg, (F) Na, (G) P, (H) S mineral yield of each genotype group under control (C), early drought (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen, old = old Swedish cultivars released from 1928 to 1990, 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same lower letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

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The Cu yield of 1R, 2R and 3R genotypes was significantly decreased by EDS while the Cu yield of most genotype groups was decreased by LDS except for modern genotypes. 3R genotypes showed a significantly higher Cu yield than modern, old, 1R and 1RS genotypes under control while modern genotypes showed a higher value than all the introgression genotypes under EDS. A higher Cu yield was noted for modern and 2R genotypes than 1R and 1RS genotypes under LDS (Fig 10B).

Old, 1R, 2R and 3R genotypes showed a decrease in K yield under EDS. LDS significantly decreased the K yield of all the genotype groups with a more severe impact on modern, old, 1RS and 3R genotypes. 3R genotypes showed a higher value than modern, 1R and 2R genotypes while old genotypes showed a higher value than modern and 2R genotypes under control. A higher K yield was found for old genotypes than 1R, 2R and 3R genotypes under EDS while a higher value was found for modern genotypes than 1R, 1RS and 3R genotypes under LDS (Fig 10C).

A decrease in Mn yield was noted for 1R and 3R genotypes under EDS while all the genotype groups showed a decrease (more profound on 1R and 3R than EDS) under LDS. 1R genotypes showed a higher Mn yield than modern genotypes under control. 2R genotypes showed a higher value than the rest of introgression genotype groups while modern genotypes showed a higher Mn yield than 1RS and 3R genotypes under LDS (Fig 10D).

Mg, Na, P and S shared the same pattern of drought effects. Only modern genotypes maintained Mg, Na, P and S yield under EDS while LDS significantly decreased the Mg, Na, P and S yield of all the genotype groups with a more severe impact on modern, old, 1R, 1RS and 3R genotypes than EDS (Fig 8E–8H). Modern genotypes showed a lower Mg yield than old and 3R genotypes under control. Old genotypes showed a higher Mg yield than all the introgression genotypes under EDS while 2R genotypes showed a higher Mg yield than 1RS and 3R genotypes (Fig 10E).

Significantly the highest Na yield was found for 3R genotypes under control. Old genotypes showed a higher value than all the introgression genotypes while modern genotypes showed a higher value than 1R and 1RS under EDS. 2R genotypes showed a higher Na yield than the other introgression genotype groups under LDS (Fig 10F).

Modern genotypes showed a lower P yield than old and 3R genotypes under control. Significantly the highest P yield was found for old genotypes under EDS while modern and 2R genotypes showed a higher P yield than 3R under LDS (Fig 10G).

Modern genotypes showed a lower S yield than old, 1R, 2R and 3R genotypes while 3R genotypes showed a higher S yield than the other introgression genotypes under control. A higher value was found for old genotypes than all the introgression genotypes under EDS while 2R genotypes showed a higher S yield than 3R genotypes (Fig 10H).

Table 1. Genotypes with high and stable grain concentration and mineral yield of eight elements (Ca, Cu, K, Mn, Mg, Na, P and S) identified by additive main effects and multiplicative interaction (AMMI).

	High and stable mineral concentration (S2 Fig)	High and stable mineral yield (S3 Fig)
Ca	258 (2R), 270 (2R), 271 (2R)	258 (2R), 270 (2R), 271 (2R)
Cu	245 (2R), 250 (3R), 251 (3R)	234 (1RS), 254 (3R), 256 (3R)
K	202 (old), 210 (1R), 215(1R), 229 (1RS), 235 (1RS)	202 (old), 203 (old), 205 (old), 207 (old), 238 (2R)
Mn	220 (1R), 222 (1R), 258 (2R)	202 (old), 203 (old), 220 (1R)
Mg	203 (old), 210 (1R), 231 (1RS), 245 (2R)	203 (old)
Na	207 (old), 256 (3R), 258 (2R)	207 (old)
P	203 (old), 210 (1R), 215 (1R), 229 (1RS), 230 (1RS).	202 (old), 203 (old), 205 (old), 234 (1RS)
S	203 (old), 210 (1R), 215 (1R)	203 (old), 234 (1RS), 238 (2R)

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Stability of Ca, Cu, K, Mn, Mg, Na, P and S

Genotypes identified by AMMI with high and stable mineral concentration (S2 Fig) and mineral yield (S3 Fig) across three treatments are listed in Table 1. Basically, Ca and Cu were dominated by 2R and 3R genotypes respectively. The high and stable concentrations of K and Mn were dominated by 1R and/or 1RS genotypes while old genotypes dominated the yield of K and Mn. No clear pattern was found for Mg and Na concentration, and only one old genotype was identified for Mg and Na yield. The high and stable concentrations of P and S were dominated by 1R and/or 1RS genotypes. Old genotypes dominated high and stable P yield while no clear pattern was found for S yield. None of the modern genotypes was identified for high stability and performance (Table 1, S2 and S3 Figs).

Discussion

This study clearly showed the contrasting performance of different sources of wheat germplasm in terms of mineral accumulation under drought stress. Genotypes with chromosome 3R demonstrated strong Zn and Fe uptake as well as high Cu, K, Na and S yield under control treatment. Several genotypes containing 3R were also identified with stable Zn and Fe accumulation from well-watered to two types of drought conditions. High and stable Se accumulation was found specifically in 1R genotypes while modern lines showed a lower accumulation in most of the studied minerals compared to other genotype groups. Old Swedish cultivars (K, Mg, Na, P and S) and introgression lines with 2R (Fe, Ca, Mn, Mg and Na) displayed outstanding tolerance in terms of mineral yield to EDS and LDS, respectively.

The significantly higher Zn and Fe accumulation found in 3R genotypes than most of the other genotype groups under control treatment suggested the presence of genes on chromosome 3R contributing to increases in the most human-health-related mineral nutrients Zn and Fe. An inadequate intake of Zn and Fe has been reported to cause a series of diseases related to liver function, diarrheal and immune system [3,4]. Interestingly, the increases in Zn and Fe yield were more significant than the increases in concentration, which might result from the high grain yield of 3R genotypes, as has been reported previously [34]. Thus, on top of the concentration increases in Zn and Fe, the total amount of Zn and Fe provided by a single wheat plant was further enhanced by positive effects on grain yield by the 3R. Furthermore, some 3R genotypes (250 and 251) were found with simultaneous high Zn and Fe levels across C, EDS and LDS conditions, proving the stability in Zn and Fe levels of these lines. The positive relationship between the grain content of Zn and Fe identified in this study, corresponds with earlier reports [38,39], thereby suggesting opportunities to breed for both these minerals in parallel. In addition to Zn and Fe, a considerably high yield of Cu, K, Na and S was also observed in 3R genotypes, which further consolidated the crucial role of 3R in increasing the nutritional value of the wheat grain and flour products. Previous studies on the functions of 3R in wheat have covered aspects such as the strengthened resistance to stem rust [40,41], grain protein content [42], tolerance to drought [34] and aluminum stress [43]. Attempts to improve grain nutrients content using chromosomes from wheat alien species have been made in a number of studies because of the two to three times higher Zn and Fe content observed in wild relatives as compared to modern wheat [44–46]. However, reports about the effect of rye chromosome 3R on the nutritional value of wheat are lacking. 3R has been reported with a significantly lower transmission rate (25.0%) than 1R (51.6%) and 2R (51.6%) during backcrossing [47], which might have hindered its wide use in wheat breeding. The potential of 3R demonstrated in this study suggests that it should be better exploited as a critical germplasm resource for the biofortification of wheat against the global problem of malnutrition (especially Zn and Fe deficiencies). In addition to the improved mineral concentration, wheat-rye introgression lines carrying chromosome 3R have also been found with a significantly higher grain protein concentration, especially compared to 1R, 1RS and 2R genotypes [42]. Effective methods should be explored to increase the rate of successful rye-to-wheat 3R transfer while genes responsible for nutritional value should be explicitly searched on chromosome 3R.

Se is another health-related essential mineral nutrient for humans and its deficiency has extended to a population of one billion worldwide [48,49] while the effect on wheat is known to be strongly dependent on the environment [50]. In this study, contrasting Se accumulation between different genotype groups was observed. Across all three treatments, 1R genotypes showed a significantly higher Se concentration than other genotype groups, except old (LDS) and 1RS, suggesting that the positive effect of chromosome 1R on grain Se concentration might withstand drought stress. This was confirmed by AMMI analysis where 1R genotypes (215, 220, 221, 222) dominated with high and stable Se concentration and yield. Rye and especially chromosome 1R, has been used as a good genetic source in wheat breeding for different purposes. Genes (e.g. *Sr31*, *Yr9*, *Lr26* and *Pm8*) present on 1R have been largely exploited for disease resistance (stem rust, stripe rust, leaf rust and powdery mildew, respectively) in wheat [51,52]. Furthermore, 1R has also been reported to be responsible for improved root traits in wheat [53,54]. In a direct comparison between wheat and rye, a 35% higher grain Se concentration was obtained in field-grown rye as compared to synthetic hexaploid and tetraploid wheat, while a 40% higher foliar Se concentration was observed in hydroponic-grown rye as compared to two wheat landraces [55]. However, for plants, Se is an unessential mineral and its function is still not clear. The uptake mechanisms of inorganic Se are related to the two major chemical forms present in soil, i.e. selenate and selenite, as these are transferred by sulfate and phosphate transporters, respectively [56,57]. The high chemical similarity between Se and S might be the reason for them sharing the same set of transporters, which further explains the widely reported interaction that Selenate and sulfate compete in the process of plant uptake [58,59]. In addition to selenite and selenite, Se also exists in organic forms in soils e.g. seleno-glutathione and seleno-methionine [60]. Despite the fact that inorganic selenate is the most bioavailable form of soil Se, wheat plants have also been shown to actively take up Se from organic sources such as seleno-methionine [60]. Although wheat is the most efficient accumulator of Se compared to other common cereal crops [61], there are no available reports about the effect of 1R on the grain Se content in wheat. From a previous study, wheat lines introgressed with 1R displayed a robust early root vigor [34] which might benefit Se uptake from soil. Unlike chromosome 3R, 1R is not facing the difficulty of the poor transmission rate. Instead, it has already been widely used in wheat breeding, although a negative effect on baking quality is often coming along with the 1R introgression in wheat [62]. Our results suggested that in addition to traits like disease resistance and yield, research focuses on chromosome 1R should be shifted to its effect on wheat nutritional value, especially Se content.

Modern genotypes were found to have generally low concentrations in most studied minerals, which correspond with earlier studies [63] and this trend suggested its compromise in nutritional value during the pursuit of high yield. At the cost of yield-oriented breeding programs, modern wheat has suffered a decline in micronutrients content relative to the landraces, alien species and wild relatives, which has been described as the dilution effect resulting from the quick yield (starch content) increase [64–66]. In this study, modern genotypes showed significantly lower Mg, P and S concentrations than old genotypes and this old-to-modern downward trend has been noted in different studies [64,67]. However, the significantly different concentrations between old and modern genotypes were only observed in Mg, P and S, which might be because all the plants were grown in pots placed in an indoor controlled-environment chamber where the more robust root system of old genotypes did not get to play a role in accessing more nutrients. A larger variation in mineral concentrations between modern and old wheat lines is expected in field conditions as has also been reported in previous studies [68,69]. In contrast, wheat-rye introgression genotypes showed a superior performance as significantly higher concentrations of Zn, Fe, Se, Ca, Cu, Mn, Mg, P and S were found in at least one of the introgressed genotype groups as compared to modern genotypes. This finding agreed with a previous study where increased levels of minerals (especially Zn and Fe) were obtained in introgressed genotypes with 1R, 2R or 5R [70]. Interestingly, the modern genotypes evaluated here also showed a lower grain protein concentration than introgressed genotypes in our previous study using a larger set (a total of 73) of genotypes [42]. Thus, rye chromosomes can be used as a strong alien genetic source to elevate both the mineral and protein contents that have been compromised in modern wheat, and therefore to fulfill the rising awareness of nutritional quality in foods.

The drastic yield decrease of wheat plants under drought stress usually consequently leads to an increase in mineral concentrations [71]. Differently, mineral yield gives the amount of minerals provided by a single plant, and therefore, it is a more suitable parameter to evaluate plants' drought tolerance in terms of minerals (mineral-yield maintaining ability). In our results, old genotypes showed outstanding tolerance to early drought due to their relatively high yield in K, Mg, Na, P and S, while 2R genotypes were found well performed in Fe, Ca, Mn, Mg and Na under late drought stress. Old wheat has been reported to show a high accumulation of minerals in several studies [64,68,72]. The genome of old Swedish cultivars might contain some ancestral genes that have been lost during decades of human selection, and reclaiming those genes gives a better opportunity in selections for tolerant lines. To our best knowledge, there are only two studies that mentioned the effect of chromosome 2R on increasing mineral concentrations in wheat [70,73]. The genomes of alien relatives to wheat are known for their broad spectrum of biotic/abiotic resistance [41,70]. Thus, with respect to mitigating the impact of drought on the nutritional value of wheat, genes responsible for tolerance to early drought and late drought should be searched in genomes of old Swedish cultivars and introgressions with chromosome 2R, respectively.

Conclusion

In the context of global climate change, food security is threatened by increasing drought events. More than two billion people across the globe are suffering from micronutrient deficiencies caused by the consumption of a nutrient-poor diet. Therefore, improvements in the nutritional value of wheat, one of the three major crops, are urgently needed. The nutritional value of wheat is largely determined by its mineral composition, especially the Zn, Fe and Se content. Here, chromosome 3R, introgressed to wheat, contributed to a high mineral yield of Zn, Fe, Cu, K, Na and S under controlled cultivation conditions, thereby demonstrating the strong role of 3R for an increase of the total amount of nutrients in wheat grown under favorable conditions. Furthermore, the 3R genotypes 250 and 251, contributed to a high and stable concentration and yield of both Zn and Fe under drought conditions, suggesting these lines as effective genetic resources to be used in breeding for high contents of both these nutrients in parallel and also for stability across climate change conditions. A high and stable performance for Se of 1R genotypes indicated a potential for the use of chromosome 1R in breeding to increase the Se efficiency of wheat. Old Swedish cultivars and introgressed 2R genotypes demonstrated tolerance to early drought and late drought, respectively, by a high mineral yield, which also resulted in significant stability across drought treatments for these lines. Thus, 3R and 1R genotypes are proposed as the two potential gene pools related to Zn/Fe and Se content across climate change environments, while old Swedish cultivars and 2R genotypes were identified as germplasms for stable mineral supplies under drought conditions. Breeding strategies should be adjusted accordingly to biofortify wheat nutritional values, as these values have been reduced in modern wheat.

Supporting information

S1 Fig. Zn (A-C) and Fe (D-E) concentration of each genotype under control (C), early drought stress (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen; old = old Swedish cultivars released from 1928 to 1990; 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R. The value of each genotype was generated from the mean of three biological replicates.
(TIF)

S2 Fig. Additive main effects and multiplicative interaction (AMMI) biplots showing concentration of (A) Ca, (B) Cu, (C) K, (D) Mn, (E) Mg, (F) Na, (G) P and (H) S versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) are those showing relatively higher stability across the three growing conditions. The vertical line in each figure indicates the average mineral concentration of the 30 genotypes.
(TIF)

S3 Fig. Additive main effects and multiplicative interaction (AMMI) biplots showing mineral yield of (A) Ca, (B) Cu, (C) K, (D) Mn, (E) Mg, (F) Na, (G) P and (H) S versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) are those showing relatively higher stability across the three growing conditions. The vertical line in each figure indicates the average mineral yield of the 30 genotypes.
(TIF)

S1 Table. Information about genotypes used in the present study.
(XLSX)

S2 Table. Data of mineral concentration, grain yield and mineral yield of each genotype.
(XLSX)

S3 Table. ANOVA table in the form of mean square values for minerals under different drought stress conditions (*: sig. < 0.001, **: sig. < 0.01, *: sig. < 0.05).**
(XLSX)

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Combating heavy metals in wheat grains under drought – is alien or ancient germplasm a solution to secure food and health?

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A B S T R A C T

Alien and ancient wheat germplasms have been utilized to combat diseases and improve yield performance under climate change. However, the potential risk of excessive heavy metal uptake with these germplasms has been less studied. In order to ensure food security, this study aimed to evaluate the levels of cadmium (Cd), lead (Pb) and mercury (Hg) in 30 wheat lines, including modern, old and wheat-rye introgression genotypes grown under three conditions i.e., control, early drought and late drought. The results of this study revealed a generally higher Cd grain accumulation in old and 1R genotypes than in the other genotype groups evaluated here, while old genotypes also showed an excess Pb grain concentration. The induced late drought resulted in an increased Cd uptake in wheat, leading to significantly elevated grain Cd concentration in modern, 1R, 1RS and 2R genotypes, while similar results were not obtained for the other heavy metals e.g. Pb or Hg. Specifically, an old genotype, 207, showed an extremely high Cd value across control and drought conditions. There was a greater genotypic variation in Pb concentration compared to Cd, while consistently high Hg concentrations were observed in several genotypes carrying 1R or 1RS. Some wheat-rye introgression genotypes, particularly those with the 3R chromosome, showed a low Cd accumulation across all treatments. The results from the present study pin-point the necessity of a rigorous assessment of heavy metal accumulation in wheat grain when utilizing ancient and alien genetic resources in breeding for disease resistance, and wheat resilience to environmental stress and climate change. Furthermore, the specific lines identified in this study with elevated heavy metal accumulation should be avoided in breeding programs. Additionally, mechanisms for the found differences in heavy metals accumulation among genotypes and treatments should be further revealed.

1. Introduction

Heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg), are metallic elements that are extremely harmful to all living organisms [1]. These elements are naturally present at various concentrations in soils throughout the earth but human industrial activities such as mining and smelting have significantly increased the environmental presence of these heavy metal pollutants [2]. Crops are known to absorb and accumulate heavy metals from the soil, posing a threat to human health, especially if staple crops consumed in large quantities by humans and animals have high levels of accumulation [3]. Wheat is one of the three major crops in the world and is primarily used as a staple food crop [4]. Field-grown wheat from various regions has been reported to accumulate heavy metals in the grains from contaminated soils [5–8]. However, significant differences in heavy metal accumulation have been observed across wheat lines indicating opportunities to breed low accumulating wheat that can thrive even in somewhat polluted soils [9,10]

Despite the fact that various novel approaches and technologies have been utilized to secure food production [11–13], climate change will significantly alter crop cultivation patterns globally. This will affect food security and the accumulation of heavy metals in food crops [14]. Drought, a common abiotic stress affecting wheat due to global warming is the most prevalent of the stresses [15,16]. Understanding the interactions between drought stress and heavy metal accumulation is crucial for safe wheat cultivation in the face of climate change [17]. Old and alien wheat material have in previous studies been found as useful resources for disease resistance [18,19], drought tolerance [20], end-use quality [21,22] and nutrition and minerals accumulation in the wheat grain [17,23]. Accumulation of heavy metals in these materials have been less studied. This study evaluates the potential of using old and alien wheat germplasm material to mitigate heavy metal accumulation (Cd, Pb and Hg) under non-drought and drought conditions using a

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diverse set of wheat genotypes. The goal of this study was to determine whether alien wheat-rye germplasm source can contribute to improved food security and production of wheat with acceptable levels of Cd, Pb and Hg. The study highlights significant variation in heavy metal accumulation in the wheat material evaluated and emphasizes the importance of avoiding 1R and ancient/old genotypes as they are potential high accumulators of heavy metals. Breeding wheat for human consumption should steer clear of such lines to prevent adverse effects on human health.

2. Materials and methods

To assess the risk of excessive heavy metal accumulation in wheat grains of different origin (modern, old and introgressed), wheat genotypes of different genetic sources were grown in climate-controlled conditions under controlled and drought stress conditions according to lan et al. (2022). Seeds were then harvested from well-watered and drought treated plants, which were subjected to heavy metal content analysis.

2.1. Plant materials and growing conditions

A total of 30 spring wheat (*Triticum aestivum* L.) genotypes grouped into modern wheat cultivars and breeding lines (n = 5), old Swedish cultivars released between 1928 and 1990 (n = 5), wheat-rye introgression lines with chromosome 1R (n = 5), 1RS (n = 5), 2R (n = 5) and 3R (n = 5) were utilized in this study (Table S1).

Plants were cultivated as previously described [20], with three biological replicates under hourly-regulated temperature and humidity based on the average climate data of Malmö, Sweden recorded from 2010 to 2019 (Swedish Meteorological and Hydrological Institute, SMHI). Three growing conditions namely control (C), early drought stress (EDS) and late drought stress (LDS) were applied.

2.2. Sample preparation

Grain samples collected at maturity from each growing condition were oven-dried (40 °C, 24 h) and milled (30 s; mixer mill 400 MM, RETSCH) into flour. All flour samples were then digested (121 °C, 200 Kpa, 30 min) with nitric acid following a similar protocol to the one previously described [24].

2.3. Heavy metal determination

All prepared sample solutions (10 ml each) underwent inductively coupled plasma mass spectrometry (ICP-MS; Aurora Elite, Bruker, U.S.) for Cd, Pb and Hg determination, with concentrations presented in mg/kg. The average amount of heavy metal contained in the grains of a single plant (μg/plant) was calculated by multiplying the concentration by the previously obtained grain yield.

2.4. Data analysis

All statistical analyses were conducted in RStudio [25]. The Cd and Pb concentrations of each genotype were visualized using R packages ‘ggplot 2’ and ‘ggbreak’. Mean comparison (LSD post-hoc test) were performed between genotype groups (modern, old, 1R, 1RS, 2R and 3R) and between treatments (C, EDS and LDS) separately using the R package ‘agricolae’. To identify genotypes with a tendency to accumulate low levels of heavy metals across the three treatments, stability analyses were performed using the additive main effects and multiplicative interaction (AMMI) with the R package ‘metan’.

3. Results and discussion

3.1. Concentration and yield of Cd, Pb and Hg

With the exception of modern genotypes under EDS, modern, 1RS, 2R and 3R genotypes, all exhibited low levels of Cd concentration in their grains. In contrast, 1R genotypes consistently maintained the highest Cd concentration among introgression genotypes across the three cultivation conditions (Fig. 1A). For the total amount of Cd provided by a single plant, modern, 1RS, 2R and 3R genotypes had significantly lower Cd yields than old and 1R genotypes under C, particularly with 1RS and 3R genotypes maintaining a low Cd yield across the three conditions (Fig. 1D). The robust and fast-developed root system conferred by the 1R chromosome likely plays a role in the higher Cd accumulation as the 1R genotypes in this study have demonstrated a more vigorous early root system than 2R and 3R genotypes [20]. Similarly, the lower Cd concentration in modern genotypes compared to old genotypes can be attributed to the smaller root system of the former. This finding alerts breeders and the food industry to the risk of elevated Cd levels in wheat products due to a larger root system. A larger root system is generally seen as a positive trait for high yield in a changing climate with drought spells. Although 1R introgression has been primarily used to enhance disease resistance [26,27] and yield performance [28] of wheat, few studies have explored the relationship between chromosome 1R and heavy metal accumulation. Therefore, the risk of excessive Cd content in grain with 1R introgression needs careful consideration in modern breeding programs and further studies are urgently needed to investigate alternatives such as transferring 1R in combination with other rye chromosomes contributing to low Cd accumulation such as 3R. Moreover, the mechanisms behind the high Cd accumulation of 1R genotypes need evaluation to avoid such behavior in drought stressed wheat varieties. Generally, it needs to be further evaluated wether a large root system such as the one in the 1R genotypes always contribute a higher uptake of everything including water, nutrients, minerals and heavy metals or if there is mechanisms that can come with the larger root system that can regulate different part of the uptake mechanisms. Such types of studies may facilitate the breeding for larger root systems and more recilient crops without the hampering effects of e.g. a high cadmium uptake.

Modern genotypes exhibited lower Pb concentration and yield than most other genotype groups under C, while, old genotypes showed the highest Pb concentration and yield under EDS (Fig. 1B and E). Significant differences in Hg concentration and yield were only observed under LDS with modern genotypes versus 1R and 1RS genotypes (Fig. 1C), and modern genotypes versus 1R (Fig. 1F), respectively. The generally low heavy metal accumulation in modern wheat aligns with the low mineral uptake of this wheat material [24]. In contrast, the high Pb accumulation in old genotypes corresponds well with a previous study that found higher Pb concentration in primitive wheat than in modern cultivars [9]. Therefore, the human-influenced evolution from old-to-modern wheat successfully reduced food toxic compounds, even though this reduction is likely due to an overall compromise in the mineral accumulation of modern wheat.

Regarding the effect of drought stress on concentrations, only LDS was found to increase Cd concentration in modern, 1R, 1RS and 2R genotypes compared to C, with no effect observed from EDS (Fig. 1A). Neither EDS nor LDS affected Pb or Hg concentrations (Fig. 1B and C). The different effects between EDS and LDS on heavy metal concentration are likely due to their different impacts on grain yield, as LDS was reported to severely reduce final yield while EDS showed no effect [20]. As for the effect on heavy metal yield, EDS decreased the Cd yield of 1R genotypes while LDS further decreased the Cd yield of modern, 1R, 1RS and 3R genotypes compared to the C condition (Fig. 1D). EDS decreased the Pb yield of 1R, 1RS and 2R genotypes and the Hg yield of old and 3R genotypes while LDS induced reductions in the Pb yield of 1R and 1RS genotypes and Hg yield of old, 1R, 1RS and 3R genotypes (Fig. 1E and F).

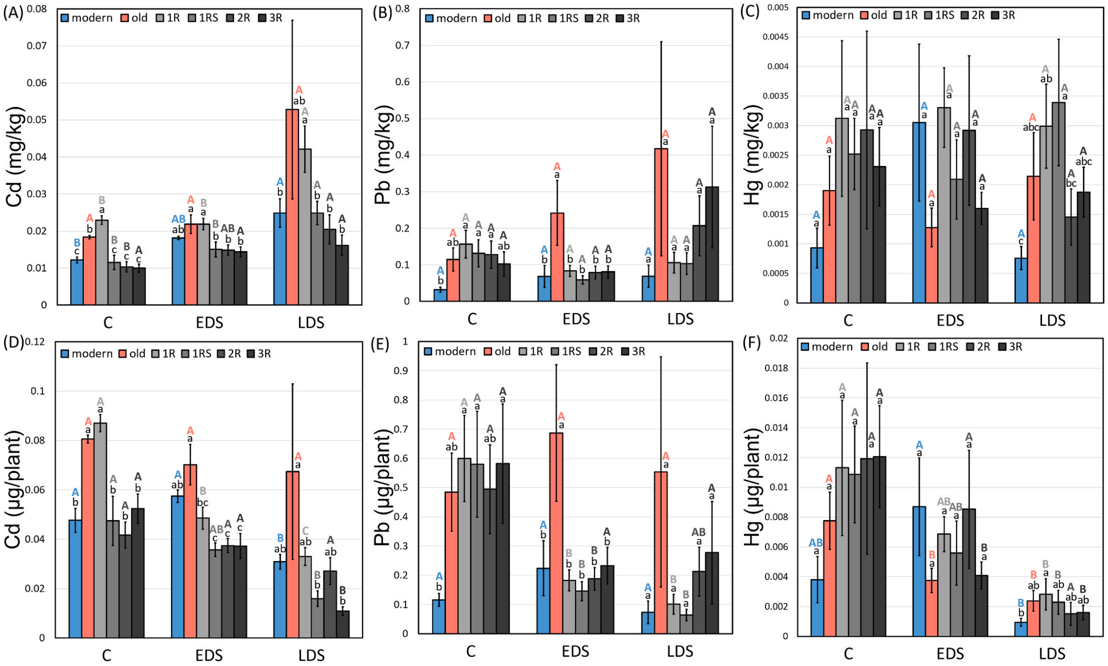

Fig. 1. The mean concentration of (A) Cd, (B) Pb and (C) Hg, and mean yield of (D) Cd, (E) Pb and (F) Hg of each genotype group under control (C), early drought stress (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen; old = old Swedish cultivars released from 1928 to 1990; 1R, 1RS, 2R and 3R = introgressions of chromosome 1R, 1RS, 2R and 3R. Means of the same genotype group between treatments marked by the same capital letters do not differ significantly. Means between different genotype groups within each treatment marked by the same smaller letters do not differ significantly (LSD post-hoc test at $p < 0.05$).

Unlike the unaffected Pb and Hg concentration, the increase in Cd concentration under LDS highlights the risk of elevated grain Cd content in the future climate. This rising trend of Cd concentration is consistent with various wheat seeds [29] and other species like peanut [30]. Thus, individually evaluating grain Cd concentration of wheat genotypes grown under different conditions becomes the major focus of this study.

3.2. Large variation and extreme values for Cd and Pb concentration

An extremely low Cd concentration was found in the 1RS genotype 235 (0.0040 mg/kg), and several other introgression genotypes; 238 (2R), 245 (2R), 250 (3R) and 256 (3R), also showed low grain Cd under C condition (Fig. 2A). The genotypes 235 (1RS), 238 (2R) and 251 (3R) had low Cd concentration under EDS while genotypes 234 (1RS), 238 (2R), 251 (3R) and 256 (3R) exhibited low Cd concentration under LDS (Fig. 2B and C). Old genotype 207 consistently showed the highest Cd concentration across C (0.077 mg/kg), EDS (0.078 mg/kg) and LDS (0.22 mg/kg) conditions, contributing to the high mean Cd concentration of the old genotype group. This highlighted several 2R and 3R genotypes as potential germplasms for reducing Cd content in wheat. In a previous study, a combination of low Cd and high Zn concentration was detected in several genotypes carrying 2R and 3R [21] and previous studies have also reported variation in Cd accumulation in various genotypes [31]. However, the potential combination of high minerals content and low heavy metals accumulation, as reported for some of the 2R and 3R genotypes evaluated here, is extremely interesting for the future breeding of highly nutritious wheat. Mechanisms behind the selected uptake of these different compounds should be further evaluated and determined in order to further elucidate breeding goals for health promoting wheat genotypes.

Unlike Cd, there was more significant genotypic variation in Pb concentration within each genotype group. A low Pb concentration was found in an old genotype, 202 (0.0047 mg/kg), and also in 235 (1RS), 248 (3R) and 279 (modern) under C conditions (Fig. 2D). Low Pb concentration was found in genotypes 202 (old), 235 (1RS), 248 (3R), 275 (modern) and 277 (modern) under EDS, while 197 (old), 202 (old), 210 (1R), 215 (1R), 238 (2R) and 279 (modern) showed low Pb concentration under LDS (Fig. 2E and F). Genotype 207 (old) with high Cd concentration also had high Pb concentration under C (Fig. 2D). Genotype 203 (old) with relatively high Cd concentration also exhibited high Pb concentration under EDS and LDS (Fig. 2E and F), suggesting a risk of elevated Cd and Pb contents under climate change. This more contrasting genotypic variation in grain Pb concentration than Cd concentration aligns well with other studies [32,33]. The low and stable Pb concentration among modern genotypes under C conditions suggested selections made in years of wheat breeding programs focusing on low Pb lines. However, those modern low-Pb genotypes did not show good consistency under early or late drought, pointing out the need for increased stability of the low-grain Pb in modern wheat to combat climate change.

3.3. Stability of concentration and yield of Cd, Pb and Hg

The additive main effects and multiplicative interaction (AMMI) clearly demonstrated different genotype × environment interactions for Cd, Pb and Hg accumulation in grains (Fig. 3). Genotypes 238 (2R), 251 (3R) and 256 (3R) were identified with low and stable Cd concentration while genotypes 235 (1RS), 250 (3R) and 251 (3R) showed low and

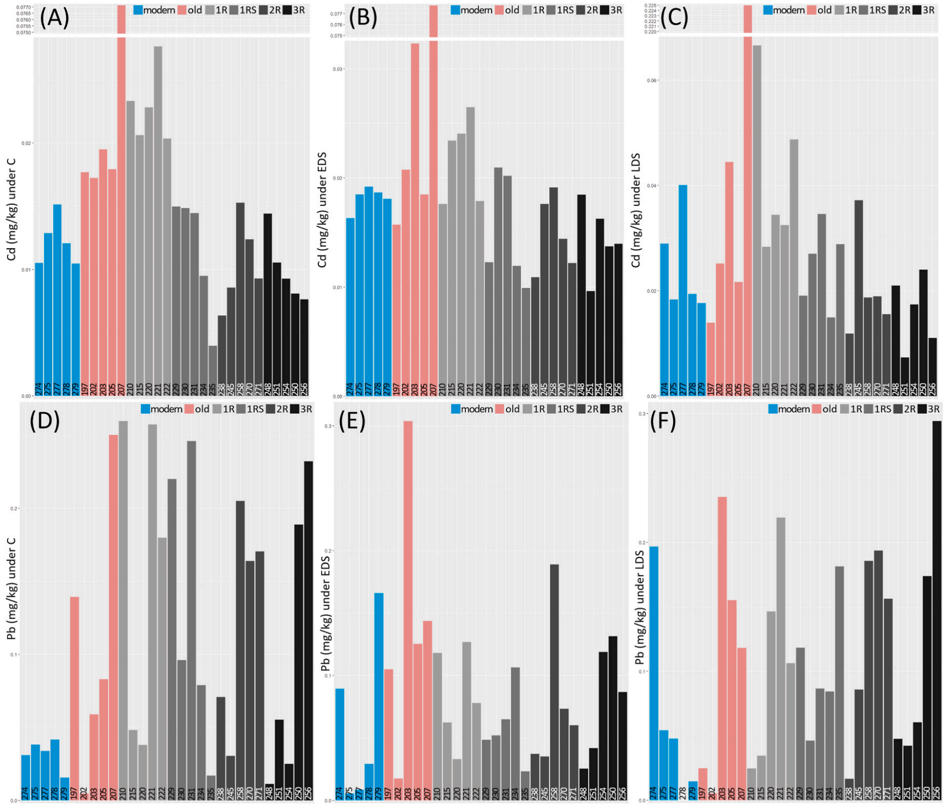

Fig. 2. Cadmium (A–C) and Pb (D–E) concentration of each genotype under control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Modern = approved cultivars and breeding lines received from company Lantmännen; old = old Swedish cultivars released from 1928 to 1990; 1R, 1RS, 2R and 3R = Introgressions of chromosome 1R, 1RS, 2R and 3R. The value of each genotype was generated from the mean of three biological replicates. The Pb concentration of genotype 278 is missing under LDS.

stable Cd yield, suggesting genes on chromosome 3R contributing to a lowered and stable grain Cd level (Fig. 3A and D). Meanwhile, old genotypes and genotypes with 1R that appeared in the above-average area suggested high Cd accumulation in their grains (Fig. 3A and D). Furthermore, the stability of this high-Cd trait for those two genotype groups across different conditions was also observed. The rapid development of an early root system in old and 1R genotypes might contribute to the high and stable Cd uptake [20], which is supported by another observation of higher Cd content in the root of an old genotype [34]. Generally, the predicted climate change will force breeders to use a wide array of novel and less adapted plant material in their strive to produce novel cultivars that will produce enough food for the growing world population. As related to the Cd results presented here, the 2R and 3R lines are promising and has the potential to contribute novel beneficial genes to the breeding gene pool, while 1R lines should be avoided. However, understanding of uptake mechanism connected to the different lines is also important for further successful use of these genes.

Considerable diversity in genetic background was found among genotypes highlighted for low and stable Pb and Hg accumulation. Two modern genotypes (275 and 277), one old genotype (202) and one 3R genotypes (248) were identified for low and stable Pb concentration (Fig. 3B). Additionally, three modern genotypes (275, 277 and 278), one old genotype (202), one 1R genotype (220), one 1RS genotype (235) and one 3R genotype genotypes (248) were identified for low and stable Pb yield (Fig. 3E). Two modern genotypes (278 and 279), one old genotype (202) and one 2R genotype (270) were identified for low and stable Hg concentration (Fig. 3C), while genotypes with low and stable Hg yield were found to be 278 (modern), 202 (old) and 270 (2R; Fig. 3F). Furthermore, genotypes with chromosome 1R (221 and 222) and 1RS (229) showed a risk of a high level of Hg (Fig. 3C). However, due to the limited scale of the current study, the low-and-stable heavy metal accumulating genotypes identified here need to be subjected to field trials of a larger scale to confirm the beneficial effects of those rye chromosomes.

4. Conclusion

The predicted climate change will have a tremendous effect on crop cultivation. To cope with the changing growing conditions with a general increase in temperature and unpridcted spells of extreme weather, currently not utilized germplasm will need to be introduced to widen the genetic base for breeding. However, introduction of novel germplasm into conventional plant breeding of food crops migh also pose human health risks at consumption cultivars developed. Human health is significantly impacted by the nutritional value and toxicity of the foods consumed daily. Intake of heavy metals from food products contribute risk of toxicity and should be avoided. Increased levels of Cd in wheat during late drought conditions suggest that wheat-based food will

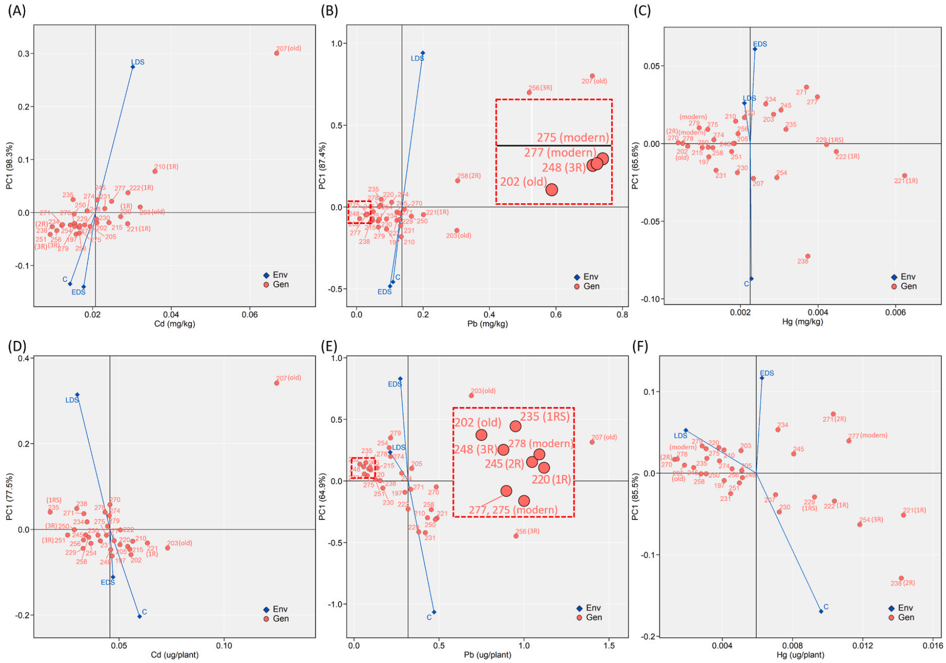

Fig. 3. Additive main effects and multiplicative interaction (AMMI) biplots showing (A) Cd, (B) Pb, (C) Hg concentration and (D) Cd, (E) Pb and (F) Hg versus the first principal component (PC1) score of 30 genotypes (Gen) and three growing conditions (Env) including control (abbreviated as C), early drought stress (EDS) and late drought stress (LDS). Genotypes located closer to the horizontal axis (score 0 on PC1) had relatively higher stability across three growing conditions. The vertical line in each figure indicates the average values of 30 genotypes. The red dashed-line square boxes indicate a zoomed-in view of the corresponding area.

potentially be higher in Cd due to climate change. This study also highlights concern of elevated Cd and Pb levels in certain wheat genotypes with chromosome 1R as well as older genotypes, such as 203, 207, 210 and 221. These genotypes are identified as potential sources of heavy metal accumulation and should be avoided in breeding programs. Some wheat-rye introgression genotypes with 1RS, 2R and 3R show promise in reducing wheat grain Cd content under both well-watered and drought conditions. The gene complexes found in the 3R lines are particularly interesting as they not only exhibited low Cd uptake, but have also shown high yield [20], high mineral content [24] and good and stable baking performance under drought conditions, which indicate their potential to be used in breeding future wheat for food security in a changing climate. The high accumulation of Cd and other heavy metals, especially in drought conditions, in the 1R genotypes, calls for more careful selection when incorporating 1R lines in breeding programs, despite their contribution to early vigor and high yield. Despite the generally high Pb content in older genotypes, specific lines like 202 with consistently low Pb levels should be further utilised as a genetic resource with low Pb content.

CRediT authorship contribution statement

Yuzhou Lan: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ramune Kuktaite:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Aakash Chawade:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Eva Johansson:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101118.

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This thesis characterised the drought tolerance of wheat using a broad set of genetic materials. Traits relating to yield, breadmaking quality, nutritional value and toxicity were investigated. The possibility of using biostimulants to enhance grain yield of wheat under drought was also explored. The novel information provided in this thesis on the effects of drought on different parameters in wheat can be applied to improve the environmental resilience of modern wheat.

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