Wild barley, a resource to optimize yield stability and quality of elite barley

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Outline

Summary

In 2050, humankind will count roughly 9.7 billion people, which is an increase of more than 2 billion people in the next 30 years. The ongoing population growth creates the mandatory need to steadily improve the efficiency of our agricultural systems to supply the world population with adequate amounts of food. The situation is even more complicated, since the improvements have to occur in spite of adverse impacts of climate change and a simultaneous reduction in arable land per person. Therefore, the plant breeding sector needs to supply farmers with more and more productive cultivars. These new cultivars have to reach higher yield levels, but also to meet the quality requirements for human and animal food. Furthermore, climate change makes it necessary to re-adapt cultivars to harsher climate conditions. Challenging in this regards is the loss of genetic variation of our crops during domestication and repetitive rounds of selection, because without variation no breeding progress is possible. Consequently, the replenishment of the elite breeding pools with new variation, especially with unrepresented alleles from wild material is a worthwhile goal.

In the following dissertation, a worldwide field study has been conducted using the wild barley population Halle Exotic Barley-YIELD (HEB-YIELD), to investigate the interplay between plant development, yield and quality under conventional agricultural conditions. The HEB-YIELD population, is a subset of the wild barley nested association mapping (NAM) population HEB-25 and consists of 48 selected HEB-25 lines. HEB-25 originated from crossing 25 diverse wild barley accessions (*Hordeum vulgare* ssp. *spontaneum* and *H.v.* ssp*. agriocrithon*) with the German elite spring barley cultivar 'Barke' (*Hordeum vulgare* ssp. *vulgare*). The 48 HEB-YIELD lines were selected based on appropriate agronomic performance (high threshability and no brittle rachis) and on combinations of allelic segregation at four major flowering time loci (*Ppd-H1, Sdw1, Vrn-H1* and *Vrn-H3*).

The test sites were Dundee (United Kingdom), Halle (Germany), Al-Karak (Jordan), Dubai (United Arab Emirates) and Adelaide (Australia), where, besides control conditions, the genotypes of HEB-YIELD have been examined under site-specific stress treatments (nitrogen deficiency in Dundee and Halle, drought in Al-Karak and Adelaide, and salt stress in Dubai). In this way, the population has been explored for the presence of genetic variation for agronomically important traits like flowering time, grain number per ear, grain yield and grain raw protein concentration. Finally, the hyperspectral imaging (HSI)

technology was tested as a possible replacement for classical wet chemistry analysis of grain quality.

The main finding was the elucidation of the complex interplay between plant development, yield and quality in an environment-specific manner. This led to the conclusion that generalizations, like the yield advantage of late maturing lines in comparison to early ones, should be treated with caution, since a detailed knowledge of the target environment is necessary to uncover the main traits and developmental phases responsible for yield formation. Furthermore, it could be shown that flowering time regulator genes (e.g. *Ppd-H1* or *Vrn-H1*) influence development throughout the whole life cycle of a plant and are potentially responsible for the environmental specific reactions. For instance, the day length-sensitive wild barley allele of *Ppd-H1* was associated with a yield increase in Al-Karak, Dubai and Adelaide, whereas in Dundee and Halle the insensitive elite barley *ppd-H1* allele had positive impacts on yield. In Al-Karak the sensitive allele improved grain yield by 5.3 dt/ha, corresponding to 30 % of the final yield level, which reflects the importance of developmental genes for yield formation.

The results of the abiotic stress treatments indicated that developmental traits are only to a lesser extent modified by the investigated abiotic stresses in comparison to yield related traits. Often, it was just a linear transformation from control to stress treatments indicating a low level of genotype by treatment interaction. The high heritability and environmental stability of developmental traits, as well as their impacts on yield and quality suits them as key traits for adaptation. Moreover, the investigated developmental regulators are defining the length of the growing period of a plant and fine tune plant developmental sub-phases (e.g. grain filling). These opportunities could be used to avoid abiotic stress situations, like terminal drought, since the combination of several of those genes could extend or shorten the growing period, as well as control the length of sub-phases.

The investigation of grain quality revealed two striking findings: First, protein and mineral concentrations in mature grains of barley are distinctively negatively correlated with yield. Second, the existence of a block of positive correlations between multiple grain ingredients pinpoints to a common uptake and final accumulation in the grain. The pronounced negative correlations are challenging for combining grain yield and grain quality into a single cultivar. Therefore, the identification of so-called correlation breaking genes are necessary to circumvent this obstacle. For example, such a locus could be

present on the short arm of 5H, where a cationic amino acid transporter is located and the wild allele increased grain ingredients without a significant negative effect on yield.
The HEB-YIELD population harbors huge amounts of variation for developmental, yield-related and quality traits, which could be directly exploited through crossing HEB-YIELD lines with elite cultivars, since the wild material is already imbedded into the genetic background of the elite line 'Barke'. The present variation for plant development can be used for a better adaptation of new cultivars to their target environment. Furthermore, the field trials revealed HEB-YIELD lines that reached or even outperformed the yield of local check cultivars, especially in Al-Karak, Dubai and Adelaide. Regarding yield performance, farmers could directly use these lines as new cultivars. Finally, the survey in such a diverse set of test sites clearly indicated the importance of knowledge about the environments for a successful adaptation, as well as the benefits of a decentralized breeding strategy, since in each environment yield formation occurred in an environment-specific manner.
HSI offers the possibility to analyze nutrient concentrations in mature grains, if the underlying calibration model, which links the measured spectra with the investigated traits, allows reliable predictions. Based on the results obtained for the nutrients N, P, K, Fe, Mg and Zn in HEB-YIELD a calibration model should not consist of less than 160 samples, which represented 40 % of the test samples used in the study, to achieve coefficient of determination values of above 0.5. Furthermore, ideally those samples should reflect the present phenotypic variation in the population(s) under survey. Since nutrient concentrations in grains are significantly influenced by year and location effects, it is recommended to expand calibration models by samples from multiple environments to improve their robustness. Moreover, the relatively simple partial least squares (PLS) regression model gave the best results in combination with the lowest computational demand. The benefits in time to analyze a sample with HSI in comparison to wet chemistry offer the application of grain quality assessments in larger populations, but with the drawback of a reduced precision in comparison to classical lab methods like inductively coupled plasma - optical emission spectrometry (ICP-OES).

Altogether, the wild barley population HEB-YIELD offers, despite its small population size, a comparable diversity than their mother population HEB-25. This fact suits HEB-YIELD beyond any doubt for the conductance of agronomic studies. Moreover, the small size in combination with the possibility to mechanical harvest enables to keep the costs for trials

on a low level. However, it should be stated that the population size makes HEB-YIELD inappropriate for in depth genetic analysis.

Zusammenfassung

Basierend auf Schätzungen der Vereinten Nationen wird die Weltbevölkerung im Jahr 2050 mehr als 9,5 Milliarden betragen, was einen Anstieg von über 2 Milliarden Menschen in den nächsten 30 Jahren bedeutet. Diese immense Zunahme der Weltbevölkerung macht es unausweichlich die Produktivität unserer Agrarsysteme zu steigern, um den erhöhten Bedarf an Nahrungsmitteln zu stillen. Erschwerend kommt hinzu, dass die Produktivitätssteigerungen vor dem Hintergrund des Klimawandels und der Abnahme der landwirtschaftlichen Nutzfläche pro Kopf erfolgen müssen. Der Pflanzenzüchtungssektor kann hierbei einen entscheidenden Beitrag leisten, durch die Bereitstellung genetisch verbesserter Sorten für Landwirte, welche vornehmlich durch gesteigerte Erträge gekennzeichnet seien sollten. Gleichzeitig müssen die Qualitätsanforderungen für die menschliche und tierische Ernährung eingehalten werden. Die bereits spür- und messbaren Einflüsse des Klimawandels erfordern eine Neuanpassung (Adaption) der Sorten an die sich veränderten Umweltbedingungen. Als problematisch für dieses Unterfangen ist der Verlust an genetischer Variation durch Domestikation und wiederkehrende Selektion zu nennen. Die Einkreuzung oder Verwendung einzelner Allele aus den Vorfahren unserer Kulturpflanzen stellt eine Möglichkeit dar zur Rückgewinnung bzw. Erhöhung der Variation in den Elitepools, da ohne das Vorhandensein von Variation kein Zuchtfortschritt möglich ist.

Die vorliegende Dissertation basiert auf weltweiten Feldversuchen mit der Wildgerstenpopulation Halle Exotic Barley-YIELD (HEB-YIELD) zur Untersuchung des Zusammenspiels zwischen Pflanzenentwicklung, Kornertrag und Kornqualität unter dem für den jeweiligen Standort üblichen Ackerbaubedingungen. Bei der Wildgerstenpopulation HEB-YIELD handelt es sich um eine Teilpopulation der "nested association mapping" (NAM) Population HEB-25, welche aus 48 ausgewählten HEB-25-Linien besteht. Die Mutterpopulation HEB-25 resultierte aus Kreuzungen zwischen 25 Wildgerstenakzessionen (*Hordeum vulgare* ssp. *spontaneum* und *H.v.* ssp*. agriocrithon*), welche aus dem Bereich des Fruchtbaren Halbmonds stammen, und der deutschen Sommergerstensorte "Barke" (*Hordeum vulgare* ssp. *vulgare*). Die 48 HEB-YIELD-Linien sind für eine gleichmäßige Segregation an vier Hauptblühgenen (*Ppd-H1*, *Sdw1*, *Vrn-H1* und *Vrn-H3*), sowie gleichzeitig guter agronomischer Eignung selektiert worden, wobei

unter agronomischer Eignung das Vorhandensein von adäquater Dreschbarkeit und die Abwesenheit von Spindelbrüchigkeit zu verstehen ist.

Die Population wurde an den Standorten Dundee (Großbritannien), Halle (Deutschland), Al-Karak (Jordanien), Dubai (Vereinigte Arabische Emirate) und Adelaide (Australien) in den Jahren 2015 und 2016 angebaut. An jedem dieser Standorte wurden die Pflanzen in einer Kontroll-, sowie Stressvariante untersucht, wobei die Stressvarianten Sickstoffmangel in Dundee und Halle, Trockenstress in Al-Karak und Adelaide, sowie Salzstress in Dubai waren. Die Population ist hierbei auf die Präsenz von Variation für züchterisch relevante Merkmale wie dem Blühzeitpunkt, der Kornzahl pro Ähre, dem Kornertrag und der Rohproteinkonzentration analysiert worden. Im letzten Teil der Dissertation steht die Evaluierung der hyperspektralen Bildgebungstechnologie (engl. hyperspectral imaging; HSI), als Ersatz zu klassischer Nasschemie für die Bestimmung von Korninhaltsstoffen im reifen Gerstenkorn, im Vordergrund.

Die zentrale Aussage der vorliegenden Arbeit ist die Aufklärung des komplexen und umweltabhängigen Zusammenspiels zwischen Pflanzenentwicklung, Kornertrag und Kornqualität. Die Ergebnisse zeigen, dass Vorsicht geboten ist bei verallgemeinernden Aussagen, wie der Ertragsüberlegenheit von späten gegenüber von frühen Sorten. Ein fundiertes Verständnis über die Zielumwelt ist notwendig, um festzustellen, welche Merkmale und Entwicklungsphasen für eine Sorte vorteilhaft sind, um den bestmöglichen Ertrag zu erzielen. Darüber hinaus konnte gezeigt werden, dass Blühgene (wie z.B. *Ppd-H1* oder *Vrn-H1*) weitaus mehr als nur den Blühzeitpunkt beeinflussen. Vielmehr handelt es sich bei ihnen um Entwicklungsgene, da sie während der gesamten Wuchsperiode Einfluss auf die Entwicklung haben und sie zum Teil für die festgestellten umweltspezifischen Reaktionen verantwortlich sind. Ein gutes Beispiel hierfür liefert das Gen *Ppd-H1*, da dessen tageslängensensitives Allel (*Ppd-H1*) in Al-Karak, Dubai und Adelaide positiv mit dem Ertrag assoziiert ist, wohingegen in Dundee und Halle das insensitive Allel (*ppd-H1*) den Ertrag erhöht. Die Bedeutung von Entwicklungsgenen für den Kornertrag wird besonders deutlich in Al-Karak, wo das sensitive Allel von *Ppd-H1* den Ertrag um 5,3 dt/ha steigert, was 30 % des finalen Ertragsniveaus entspricht.

Die Auswertung der Ergebnisse der abiotischen Stressbehandlungen brachten zum Vorschein, dass Entwicklungsmerkmale im Vergleich zu ertragsrelevanten Merkmalen nur geringfügig durch abiotischen Stress beeinflusst sind. Zum Teil handelte es sich dabei um eine nahezu lineare Transformation zwischen Kontroll- und Stressvariante, was durch hohe positive Korrelationen zwischen den Behandlungen, sowie dem Fehlen von

Genotyp×Behandlungs-Interaktionseffekten aufgezeigt wurde. Die Kombination aus hoher Heritabilität, Umweltstabilität, sowie deren Einfluss auf Ertrag und Qualität macht Entwicklungsmerkmale zu Schlüsselmerkmalen für die Adaption. Vielmehr noch bestimmen sie die Länge der Wachstumsphase, als auch die Länge der einzelnen Subphasen (z.B. Schossen) der Pflanzenentwicklung. Dies ermöglicht durch die richtige Kombination von mehreren Entwicklungsgenen die Vermeidung von abiotischen Stresssituationen, besonders zu solchen Phasen, die maßgeblich für den Kornertrag sind. Hierfür kann beispielhaft der Blühzeitpunkt oder die Kornfüllungsphase genannt werden. Bei der Analyse der Makro- und Mikronähstoffe im reifen Gerstenkorn konnten zwei wesentliche Beobachtungen gemacht werden. Zum einen, das Vorhandensein einer ausgeprägten negativen Korrelation zwischen Protein- und Nährstoffkonzentrationen mit dem Ertrag, sowie zum anderen, die Existenz eines positiven Korrelationsblocks zwischen den Kornnährstoffen. Letzteres weist auf eine gemeinsame Aufnahme und Akkumulation im Korn hin. Die negative Korrelation zwischen Konzentration und Ertrag macht es schwierig hohe Nährstoffkonzentrationen mit hohem Kornertrag in einer Sorte zu verbinden. Aus diesem Grund ist die Identifikation von Genorten, die als sogenannte Korrelationsbrecher fungieren, welche sich positiv auf die Nährstoffkonzentration auswirken ohne gleichzeitig einen negativen Effekt auf den Ertrag zu haben, entscheidend, um das oben beschriebene Hindernis zu überwinden. Ein solcher Genort könnte auf dem kurzen Arm von Chromosom 5H liegen, wo sich ein kationischer Aminosäuretransporter befindet und das Wildallel die Kornnährstoffkonzentrationen erhöht ohne einen signifikant negativen Effekt auf den Ertrag auszuüben.

Zusammenfassend lässt sich festhalten, dass die Wildgerstenpopulation HEB-YIELD mit einer bedeutsamen Variation für Entwicklungs-, Ertrags- und Qualitätsmerkmale aufwartet. Diese Variation kann zügig nutzbar gemacht werden, da die Wildlinien durch Kreuzung und Rückkreuzung mit dem Eliteelter Barke in dessen genetischen Hintergrund eingebettet sind. Ein mögliches Szenario ist die Verwendung der Variation, um zukünftige Sorten besser an die Umweltbedingungen in ihren Zielumwelten anzupassen. Darüber hinaus konnten einige HEB-YIELD Linien das Ertragsniveau der Standards in den Standorten Al-Karak, Dubai und Adelaide erreichen oder gar übertreffen, was die Chance bietet, diese Linien direkt in der Landwirtschaft zu verwenden. Die Durchführung einer Studie in solch diversen Umwelten hat deutlich gezeigt, wie wichtig es ist, ein umfassendes Verständnis über die Umweltbedingungen (wie Klima, Boden und Wetterextreme) zu haben, um eine bestmögliche Adaption an die Zielumwelt zu

ermöglichen. Des Weiteren konnte der Vorteil einer dezentralen Züchtungsstrategie aufgezeigt werden, da die Ertragsbildung an jedem der untersuchten Standorte signifikant durch die vorherrschenden Umweltbedingungen beeinflusst wurde.
Die HSI-Technologie ermöglicht die Messung von Makro- und Mikronährstoffen im reifen Gerstenkorn, wenn das zu Grunde liegende Kalibrationsmodell, welches die gemessenen Spektren mit dem eigentlichen Merkmal verbindet, vertrauenswürdige Schätzungen erlaubt. Die Ergebnisse aus HEB-YIELD zeigen, dass ein Kalibrationsmodell für die Messung von N, P, K, Fe, Mg und Zn mindestens 160 Proben, oder in anderen Worten 40 % der gesamten Stichproben, umfassen sollte, um Bestimmtheitsmaßwerte von über 0,5 zu erhalten. Aus bereits veröffentlichten Arbeiten ist bekannt, dass Korninhaltsstoffe durch Jahr- und Ortseffekte beeinflusst werden. Dies konnte durch die vorliegenden Resultate bestätigt werden, aus welchem Grund Kalibrationsmodelle aus Daten von mehreren Umwelten bestehen sollten, um ihre Robustheit zu erhöhen. Das „partial least squares“ (PLS) Regressionsmodell konnte trotz seiner relativen Einfachheit im Vergleich zu artifiziellen neuronalen Netzwerken die besten Schätzungen liefern, in Verbindung mit dem niedrigsten Rechenbedarf. Der entscheidende Vorteil der Bestimmung von Korninhaltsstoffen mittels HSI ist die Möglichkeit, eine große Zahl an Proben im Hochdurchsatz zu messen. Es muss allerdings erwähnt werden, dass es technisch bedingt zu einem Verlust an Genauigkeit im Vergleich zu nasschemischen Methoden wie „inductively coupled plasma - optical emission spectrometry“ (ICP-OES) kommt.

Abschließend lässt sich festhalten, dass die Wildgerstenpopulation HEB-YIELD, trotz ihrer geringen Populationsgröße, eine annähernd vergleichbare Diversität wie ihre Mutterpopulation HEB-25 aufweist und somit zweifellos für die Durchführung von agronomischen Studien geeignet ist. Die geringe Größe und die Möglichkeit der maschinellen Ernte bieten den Vorteil, dass Versuche mit der HEB-YIELD ohne großen Kostenaufwand möglich sind. Andererseits muss erwähnt werden, dass aufgrund der Populationsgröße, die HEB-YIELD ungeeignet ist für tiefgreifende genetische Untersuchungen.

I. General introduction

I.I. Prospective challenges of agriculture

Agriculture was the basis for the emergence of sedentary human civilizations, which is still forming our modern life (Flannery 1973; Mazoyer and Roudart 2006). Agriculture provides us with our daily food, animal forage, bioenergy and raw materials as well as medicine (Power 2010), whereupon cereals play a major role as they account for 50 % of the global needed calories (Kearney 2010; OECD and FAO 2017). Agriculture always had to face challenges since its dawn more than 10,000 years ago. However, to feed a world population of more than 7 billion people, which is still growing, creates high pressure to further efficiency improvements of our cropping systems (Godfray et al. 2010; OECD and FAO 2017). The rise in production needs to occur despite increasing impairments of climate change and a reduction in arable land per person (Godfray et al. 2010; Powell et al. 2013). Different climate prediction models indicate heavy impacts of climate change in large parts of Africa, the Arabian Peninsula, Southeast Asia and Central South America (Fig. 1; Samson et al. 2011; Field et al. 2014).

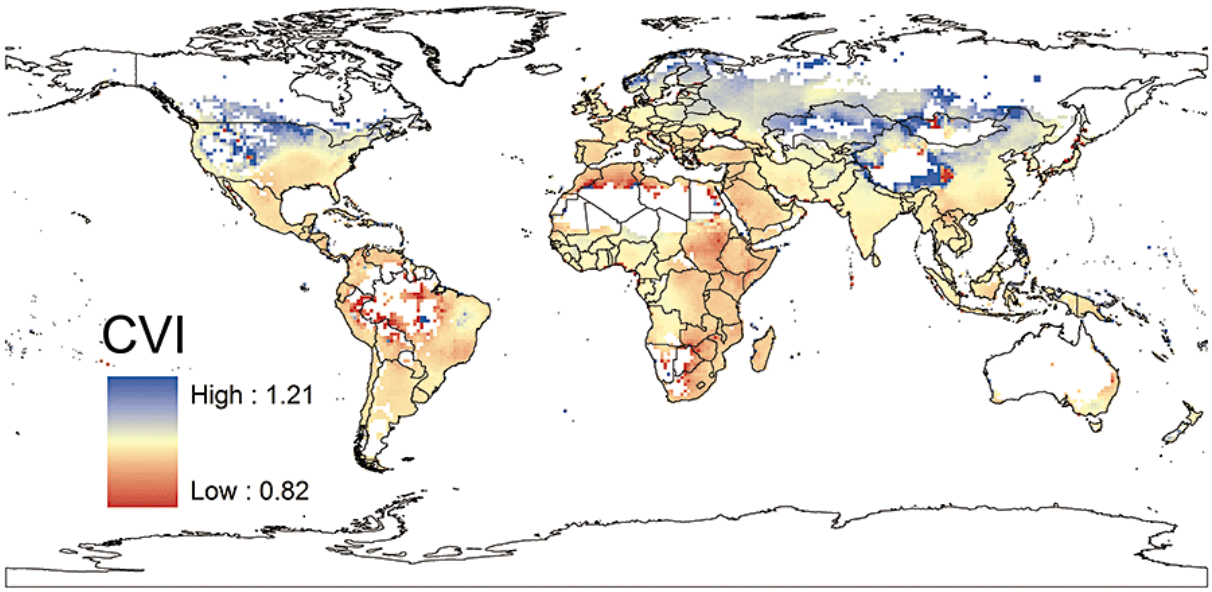

Figure 1 | Geographic disparities in the predicted impacts of climate change on human populations

Climate vulnerabilities index (CVI) expressed as climate-consistent annual growth rate based on current human density–climate relationships and a 2050 climate forecast. Climate-consistent annual growth rates of less than one, indicated in red, represent negative growth and high vulnerabilities, while changes in annual growth rates of greater than one, indicated in blue, represent positive growth and low vulnerabilities. White regions correspond to human density values of zero in the global gridded population database (Samson et al. 2011).

The fifth assessment report of the intergovernmental panel on climate change (IPCC) showed that a multiplicity of studies detected negative impacts of climate change on crop yields (Fig. 2; Field et al. 2014). Their findings suggest that the mean yield of our crops will be reduced by around 1 % per additional decade due to climate change. Alexandratos and Bruinsma (2012) reported that until 2050 a yield increase of roughly 14 % per decade is necessary to fit the increasing demands. Therefore, future yields need to reach this level while compensating for yield losses through harsher environmental conditions - especially drought and salt stress are a threat for large agricultural production areas (Bartels and Sunkar 2005; Mahajan and Tuteja 2005). These threats will make it mandatory to adapt our crops to the new environmental conditions (Ceccarelli et al. 2010; Tester and Langridge 2010; Powell et al. 2013). The positive effects of adaptation to handle the impacts of climate change were recently highlighted by the IPCC report, where they showed significantly lower yield reductions in temperate regions if crops were well adapted to their environment (Field et al. 2014).

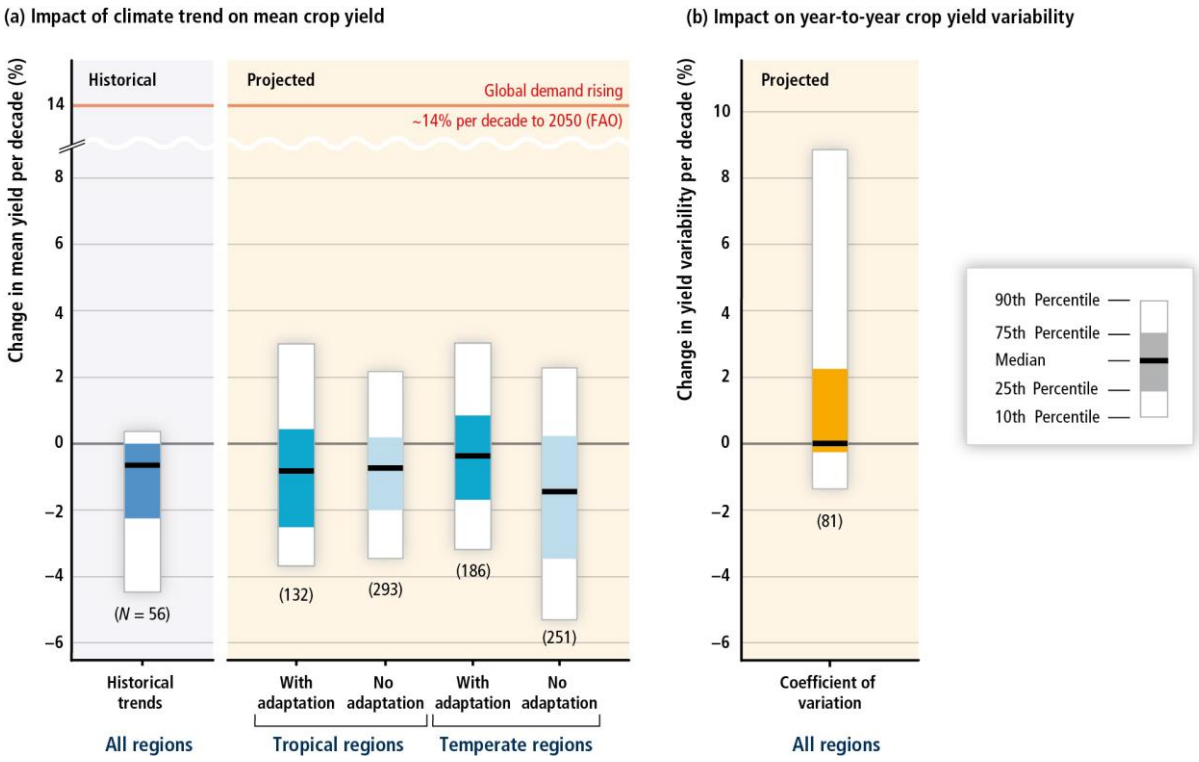

Figure 2 | Impacts of climate change on crop yields

Boxplot summary of studies that quantify impact of climate and CO_2 changes on crop yields, including historical and projected impacts, mean and variability of yields, and for all available crops in temperate and tropical regions. All impacts are expressed as average impact per decade (a 10 % total impact from a 50-year period of climate change would be represented as 2 % per decade). N indicates the number of estimates, with some studies providing multiple estimates. In general, decreases in mean yields and increases in yield variability are considered negative outcomes for food security. Also indicated in the figure is the expected increase in crop demand of 14 % per decade (Alexandratos and Bruinsma 2012), which represents a target for productivity improvements to keep pace with demand (Field et al. 2014).

I.II. Barley - crop number four

The importance of cereals, in particular maize, rice, wheat and barley, as staple food for the majority of humankind has already been mentioned. Barley (*Hordeum vulgare* ssp. *vulgare*) is in regard to production the fourth most important cereal crop on a global scale, whereupon Russia, France and Germany are the top three producers (Fig. 3; Zhou 2010; FAOSTAT 2017).

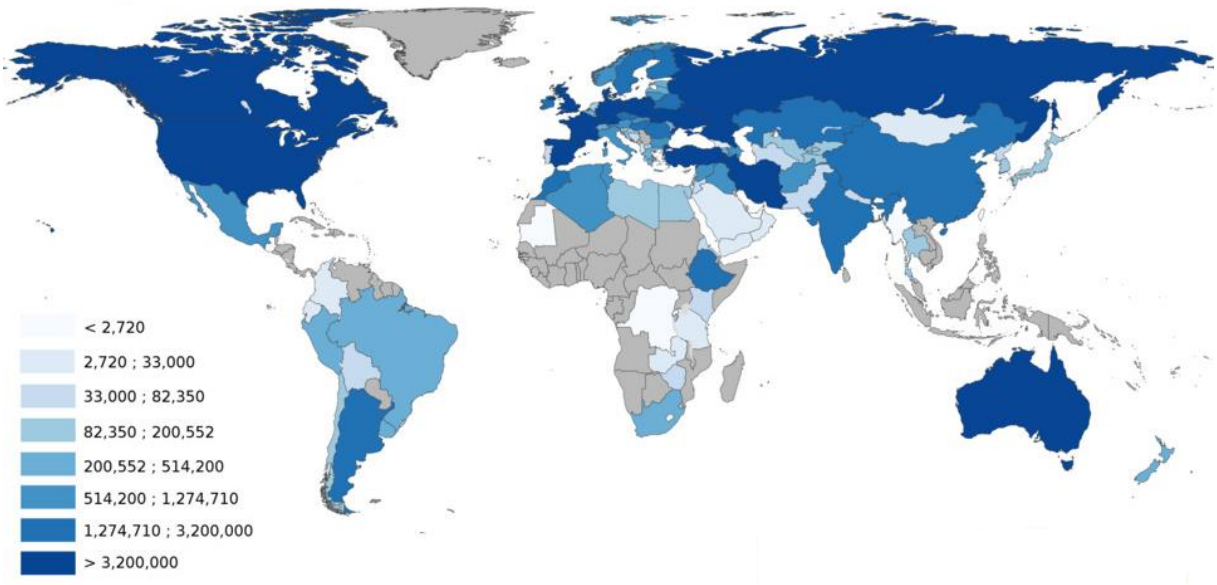

Figure 3 | World map of barley production - 2014 (in tons)
The countries are colored based on their barley production in tons from 2014, whereupon a grey color indicates no available data. The production values are based on the Food and Agriculture organization of the United Nations (FAO) from 2014 (Actualitix).

The lion's share of barley is used as animal fodder, especially as source for carbohydrates and proteins for ruminants and pigs (Zhou 2010; Wrigley et al. 2017). Number two in line of barley consumption is the beverage industry using malt for producing beer and whisky (Zhou 2010; Wrigley et al. 2017). Smaller amounts of this ancient crop are used as human food, notably in parts of Africa, the Near East, the highlands of Central Asia, the Andean countries and the Baltic states where it still plays an important role for human nutrition (Zhou 2010). It should be mentioned that during the last years there was a small but steady increase as human food, because of the beneficial nutritional value of barley grains (Baik and Ullrich 2008; Baik et al. 2011; Wrigley et al. 2017).

In comparison to the major cereal wheat, barley exhibits two essential advantages. Firstly, diploid genetics (Sreenivasulu et al. 2008) and secondly, a higher tolerance versus abiotic stresses (Munns and Tester 2008; Baik et al. 2011; Nevo et al. 2012). The simple genetics

in combination with the tight relationship among the members of the *Triticeae* tribe (e.g. soft wheat, durum wheat and rye) suits barley as an ideal model to transfer genetic knowledge gained in barley to other *Triticeae* species (Sreenivasulu et al. 2008). In addition, the higher abiotic stress tolerance might be a useful characteristic to extend barley production in those areas that are heavily affected by climate change (Fig. 1), providing a secure source for human food.

I.III. Plant developmental regulation in barley

Like many other cereals barley can be separated into spring and winter types, depending on the absence or presence of the vernalization requirement, respectively. Vernalization occurs through the exposure of plants to cold temperatures for a genotype specific period, which is necessary to induce flowering (Trevaskis et al. 2007; Rollins et al. 2013). Flowering time (also known as heading date) is concisely regulated by environmental cues like day length (photoperiod) and the above mentioned vernalization, as well as an ambient temperature in general (Andrés and Coupland 2012; Fjellheim et al. 2014). Moreover, there are several additional parameters controlling flowering time such as the circadian rhythm (Campoli et al. 2012 ;Johansson and Staiger 2015), the nutrient supply, abiotic and biotic stresses (Cho et al. 2017) and phytohormones like gibberellic acid (Mutasa-Göttgens and Hedden 2009; Boden et al. 2014). Nevertheless, flowering time is under tight genetic control and highly heritable, indicated by the identification of a multiplicity of genes controlling the trait (Blümel et al. 2015). A simplified scheme of the flowering time network in wheat and barley is given in Figure 4, where the photoperiod and vernalization pathway are depicted.

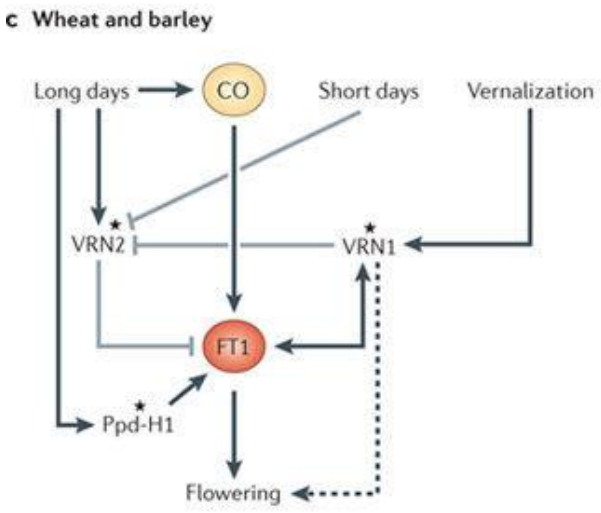

Figure 4 | Flowering time regulation in wheat and barley

In the temperate cereals wheat and barley, flowering is promoted by long days. *CONSTANS (CO)*-like proteins are involved in the activation of *FLOWERING LOCUS T* (*FT)*-like genes in response to long days; however, the pseudo-response regulator *Ppd-H1* is also required for transcriptional activation of *FT*-like genes under long days. Vernalization is required to accelerate flowering in these species. During vernalization, the transcription of *VERNALIZATION 1* (*VRN1*) is increased. *VRN1* promotes inflorescence development and represses transcription of *VRN2*. *VRN2* blocks expression under long days of at least one of the *FT*-like genes in these species (namely, *FT1*), and its expression is repressed during the winter by vernalization via *VRN1*. Exposure to short days also represses *VRN2*, allowing *FT1* expression, which promotes flowering in summer (figure adapted from figure 2 in reference Andrés and Coupland 2012).

Ppd-H1 is the main regulator of photoperiodism in barley and the dominant allele (= responsive to day length) upregulates the expression of *Vrn-H3*/*HvFT1* (*FT1*) under long day conditions. In most cases winter and wild barleys carry the dominant allele, whereas spring barleys possess the recessive non-responsive *ppd-H1* allele, resulting in a later flowering (Turner et al. 2005; Johansson and Staiger 2015). The vernalization pathway starts with the recognition of cold temperatures by the *MADS-box* transcription factor gene *Vrn-H1* (*VRN1; Oliver et al. 2013*), whose expression gets upregulated by cold temperatures (Yan et al. 2003). The higher transcripts of *Vrn-H1* down-regulates the flower repressor *Vrn-H2* (*VRN2*), which prevents the induction of flowering before the end of winter in winter barley cultivars and wild barleys (Yan et al. 2004). On the other hand, spring barley cultivars are characterized by a natural deletion of *Vrn-H2* and a dominant or semidominant allele at the *Vrn-H1* locus, ending in the absence of the vernalization requirement (Casao et al. 2011). In addition, *Vrn-H1* promotes the expression of the central flowering promoter *Vrn-H3* or *FT1*, the Flowering locus T in *Arabidopsis* (Deng et al. 2015), which integrates photoperiod and vernalization signals (Loscos et al. 2014). By raising the transcript levels of *Vrn-H3*, the flower induction gets accelerated through the expressional activation of meristem identity genes (like *Vrn-H1*

in barley or *APETALA1* in Arabidopsis) in the shoot apical meristem, which are fulfilling the switch to generative growth (Andrés and Coupland 2012; Fjellheim et al. 2014).

The already mentioned gibberellic acid (GA) network is an additional regulator of flowering time in plants, since the level of active GA controls the progress of GA dependent growth processes (Mutasa-Göttgens and Hedden 2009). The semi-dwarf growth type in barley and wheat is the result of an impaired GA network, which is governed by the group of so-called semi-dwarfing genes (Flintham et al. 1997 ;Kuczyńska et al. 2013). In barley *Sdw1* (also called *denso*) is the main semi-dwarfing locus (Kuczyńska et al. 2013) and responsible for height reductions of more than 20 cm (Hellewell et al. 2000). Most modern barley cultivars possess a semi-dwarf phenotype as a result of non-functional and recessive alleles at the *Sdw1* locus, which are coding for a gibberellic acid 20 oxidase *(GA20ox)* gene (Jia et al. 2009; Jia et al. 2015). Such genotypes are defined as GA deficient, because there GA level is considerably lower than those of tall plants carrying a functional allele of *GA20ox* that catalyzes a step in the synthesis of active GA (Jia et al. 2009; Jia et al. 2015). The reduction in the amount of GA slows down the growth processes, which delays the time point of flowering (Kuczyńska et al. 2013).

Several studies in barley revealed that flowering time regulator genes exhibit impacts throughout the whole plant development, indicating that these genes are not only responsible for flower induction (Huijser and Schmid 2011; Maurer et al. 2016; Herzig et al. 2018; Wiegmann et al. 2019a)

I.IV. Impacts of plant development on crop fitness

Grain yield is the most complex plant trait and the gold standard for selecting superior crop cultivars by breeders and farmers. Disregarding the influence of a multitude of factors like climate, plant development, resistance versus pathogens and tolerance versus abiotic stresses, grain yield can also be explained as the product of yield components (Fig. 5), mainly the number of spikes per square meter, the number of grains per spike and the grain size (Slafer 2003). However, it should be noted that this is an oversimplification and ignores the fact that most yield components are negatively correlated among each other (Slafer 2003; Slafer et al. 2014).

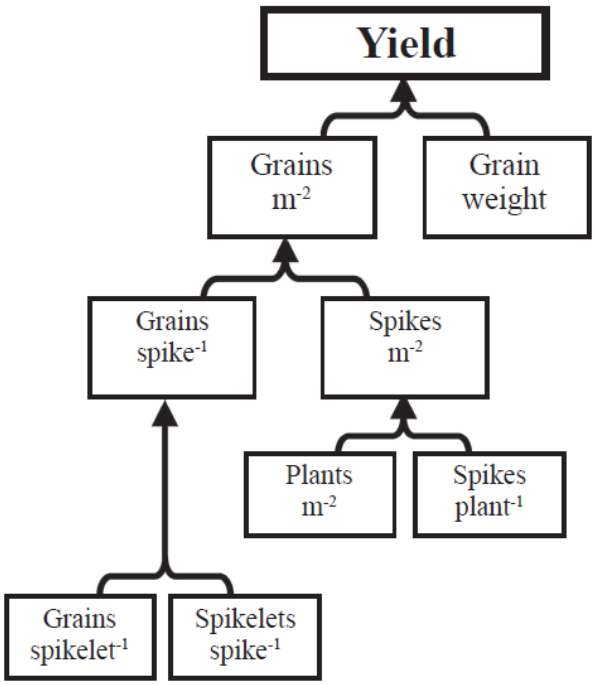

Figure 5 | Composition of grain yield in cereals
Diagram of cereal yield illustrating the relationships between the various yield components commonly measured in agronomic trials (figure adapted from figure 3 in reference Slafer 2003).

Every plant passes through several developmental stages during its life cycle, where in each stage a specific set of traits develops, including yield components (Fig. 6; Slafer 2003). One of these stages is flowering time, which is a key event since plants switch from vegetative to generative growth, to operate the turnout on yield formation (Worland 1996; Cockram et al. 2007; Kamran et al. 2014). Therefore, this stage should occur in the absence of abiotic stresses, like drought or frost (Slafer et al. 2009; Kazan and Lyons 2016). Another example would be the morphogenesis of the spike, separated into spikelet initiation, spikelet abortion and spike growth, where the final number of grains per area is defined (Sreenivasulu and Schnurbusch 2012). The latter one is the key yield component, since every increase in grain number should finally increase grain yield (Miralles et al. 2000; Sreenivasulu and Schnurbusch 2012; Slafer et al. 2014). This developmental phase is genetically also influenced by flowering time regulators (Sreenivasulu and Schnurbusch 2012; Zhang and Yuan 2014) and the right timing improves spikelet and floret fertility (Serrago et al. 2008; Sreenivasulu and Schnurbusch 2012).

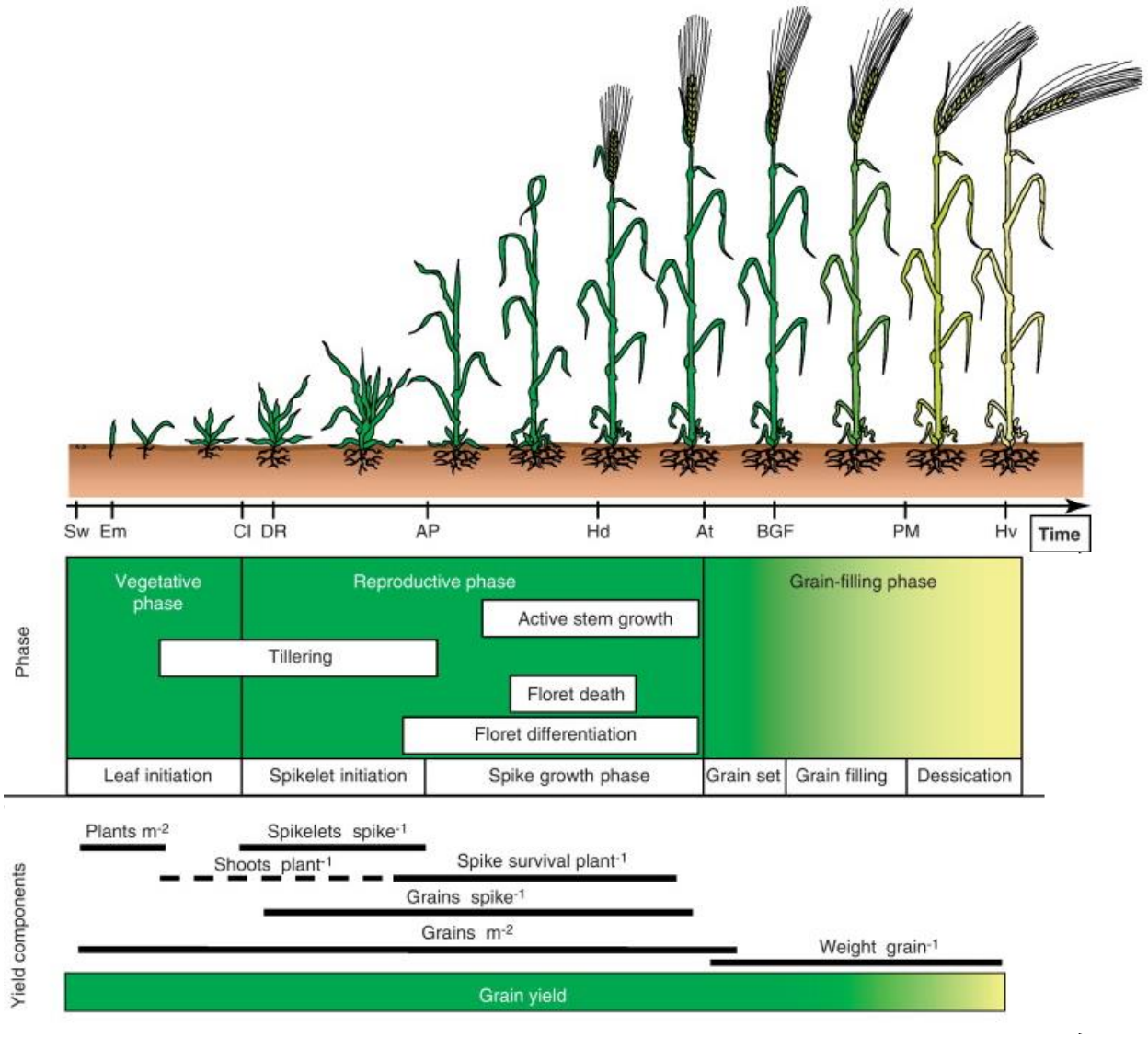

Figure 6 | Plant development and yield formation in cereals

The major phases of barley development and their temporal occurrence in relation to the establishment of components of grain yield. The bars in the lower subplot indicate the onset and end of the respective yield component. Abbreviations: Sw = sowing; Em = seedling emergence; CI = collar initiation; DR = double ridge; AP = awn primordium; Hd = heading time; At = anthesis; BGF = begin grain filling; PM = physiological maturity; Hv = harvest (figure adapted from figure 3 in reference Sreenivasulu and Schnurbusch 2012).

Moreover, the final length of the growing period of a plant from sowing until harvest defines the yield level of a crop. In areas where the environmental conditions support late maturing lines a long growing period positively affects yield (Cockram et al. 2007; Sacks and Kucharik 2011; Alvarez Prado et al. 2017), whereas in areas that are affected by terminal drought and heat stress earliness is associated with higher yields (Shakhatreh et al. 2001; Al-Ajlouni et al. 2016).

Overall the precise timing of the occurrence of plant developmental stages, as well as defining the length of the growing period offers the possibility to escape abiotic stress situations, resulting in improved grain yields (Kazan and Lyons 2016).

I.V. Quantity versus quality - a dilemma

Since the beginning of the "Green Revolution" yield enhancements of our main crops had tremendous success, especially in developing countries (Gaud 1968; Evenson and Gollin 2003). However, cereals like barley do not only provide carbohydrates to human food and animal fodder. They are also a source for proteins, fiber and nutrients (Fig. 7; McKevith 2004; Gaudichon 2015; Wrigley et al. 2017), especially in countries where incomes fall below the costs of animal based products (Tilman et al. 2011; OECD and FAO 2017). Therefore, high yields have one dramatic drawback - they may result in a reduction in grain quality, indicated by lower protein and nutrient concentration of grains. This in turn may result in lower nutritional values of the food and fodder produced from them (Simmonds 1995; Oury et al. 2003; Fan et al. 2008). The loss of nutritional value is mainly the result of the so called "dilution effect", which occurs by raising grain yield (Kibite and Evans 1984; Arnon 1992; Simmonds 1995; Murphy et al. 2008; Guttieri et al. 2015). Crop yields can be improved in different ways, for instance through heavier grains (Slafer 2003; Slafer et al. 2014), as a result of more starch accumulation in the endosperm (Jenner et al. 1991; Beckles and Thitisaksakul 2014). However, proteins and minerals are mainly stored in the aleurone layer surrounding the endosperm (Fig. 7; Shewry et al. 2013). Therefore, an increase in grain yield results in a distribution of grain ingredients into more or heavier grains, causing a dilution effect, because relatively small amounts of proteins and minerals are stored in comparison to the enlarged starchy endosperm.

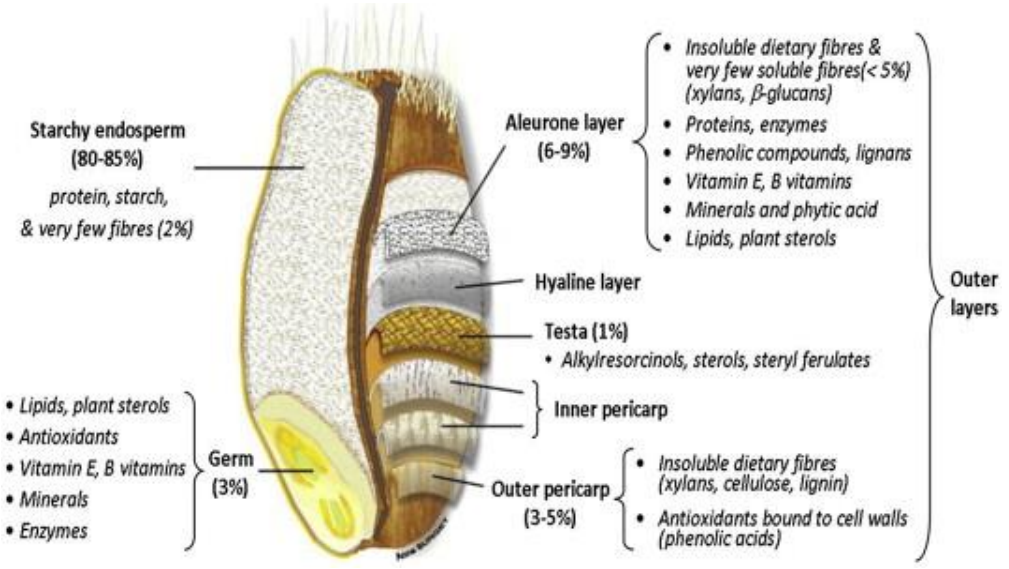

Figure 7 | The wheat and barley grain

Indicated are the different grain components and their percentage of the whole grain weight. Moreover, a detailed ingredient composition of each component is given (Brouns et al. 2013).

The importance of providing mankind with high quality food products is obvious. Round about one billion people suffer from low intakes of proteins and nutrients, especially iron, zinc and calcium (White and Broadley 2009; Carvalho and Vasconcelos 2013; Wu et al. 2014). Usually, plants do not accumulate proteins and nutrients for human consumption, instead they do it for their own specific needs to ensure a sound plant development and survival of the next generation (Dimkpa and Bindraban 2016). In the past, numerous studies have been conducted to identify genotypes or genetic variation for the re-improvement of the nutritional value of modern crop cultivars, often termed as "biofortification" (Zhao and McGrath 2009; Jiang et al. 2009; Carvalho and Vasconcelos 2013; Guttieri et al. 2015; Pandey et al. 2016; Soleimani et al. 2017). Nevertheless, so far the success of these approaches was unsatisfactory (Bogard et al. 2010). One obstacle is the pronounced negative relationship between yield and protein or nutrient concentration (Simmonds 1995; Murphy et al. 2008; Acreche and Slafer 2009; Zhao et al. 2009; Guttieri et al. 2015), meaning that an increase in nutritional value is frequently accompanied by a reduction in yield.

I.VI. Grain quality analysis - wet chemistry versus spectroscopy

Traditionally, the quality of cereal products has been measured based on wet chemistry analysis, such as the determination of the protein concentration of grains, as well as the digestibility of animal fodder, for instance using combustion analysis and/or inductively coupled plasma - optical emission spectrometry (ICP-OES). The methods of wet chemistry are well-established and frequently used, however they are time-consuming, labor-intensive and expensive (Foley et al. 1998; Stuth et al. 2003; Spielbauer et al. 2009). Altogether, this makes them inapplicable for high-throughput analysis of large test samples, which is often required in plant breeding (Osborne 2006; Diepenbrock and Gore 2015). In the last decades, spectroscopy technologies, notably near-infrared reflectance spectroscopy (NIRS) have been widely applied in quantitative and qualitative analysis of organic compounds (Foley et al. 1998; Montes et al. 2007). They circumvent the above mentioned disadvantages and represent a non-destructive method, which suits them for analysis of plant seeds (Foley et al. 1998; Spielbauer et al. 2009). NIRS is already an established technology in plant breeding (Montes et al. 2007; Pojić and Mastilović 2013), where it is for example routinely used to estimate protein and starch concentration of

maize kernels, as well as digestibility of maize silage (Barrière et al. 1997; Stuth et al. 2003). The basic principle of spectroscopy technologies is the irradiation of a sample with a specific wavelength (e.g. near-infrared, 750-2500 nm), which gets transmitted, absorbed and reflected by the sample. The reflected radiation is recorded by detector units. The specific spectrum (Fig. 8) contains information about the physical and chemical characteristics of the sample, where the reflection spectrum can be regarded as a chemical fingerprint. Finally, the reflection spectrum gets converted into phenotypic values by using calibration models, which can be treated like conventional values obtained from wet chemistry (for more details about NIRS see Foley et al. (1998) and Cen and He (2007).

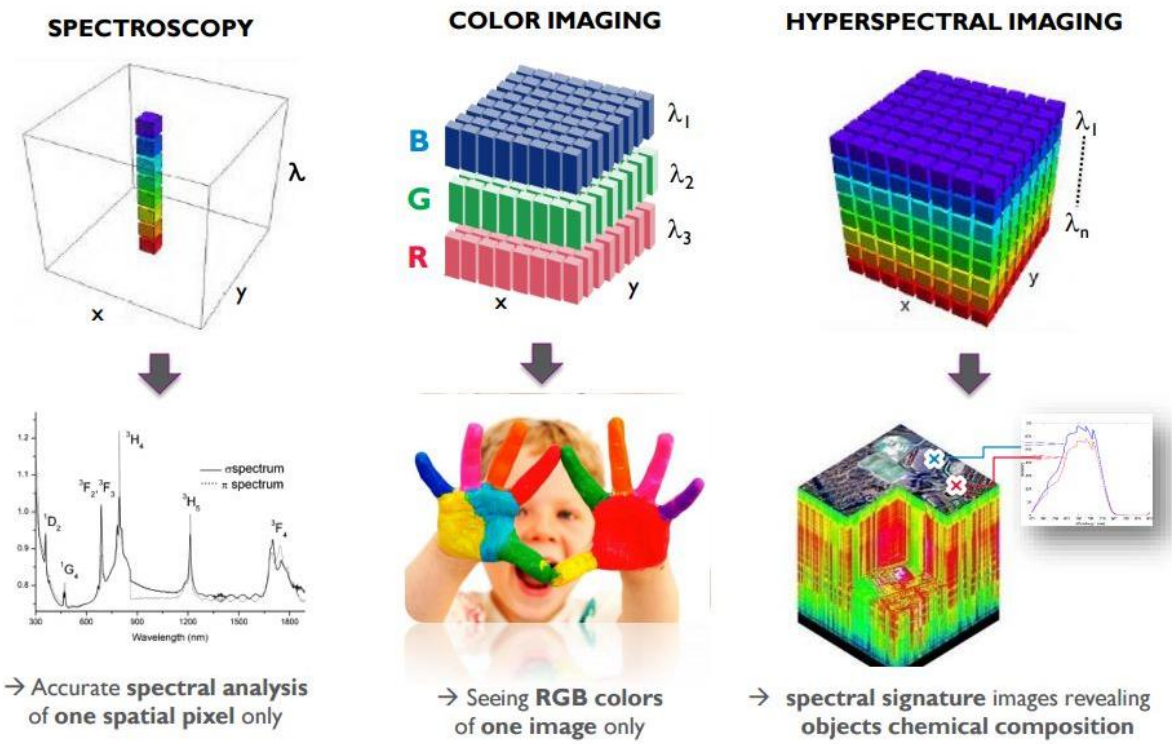

Figure 8 | Comparison between spectroscopy, color imaging and hyperspectral imaging
Figure adapted from © imec 2015, https://www.imec-int.com/en/hyperspectral-imaging.

A related technology is hyperspectral imaging (HSI), which combines spectroscopic and vision techniques (ElMasry and Sun 2010; Park and Lu 2015). The main advantage in comparison to spectroscopy-based technologies is that hyperspectral imaging additionally gives spatial information about a sample, such as the allocation of a specific chemical component in a sample. Therefore, hyperspectral images can be considered as three-dimensional data cubes, since they are composed of a two-dimensional image and spectral data, which is forming the third dimension (Fig. 8; Chao et al. 2001). The image provides the allocation of the chemical components in the sampled object and the spectral data is used to identify the components based on their spectrum (for more details about

HSI see ElMasry and Sun (2010) and Park and Lu (2015)). This technology has already been successfully applied in a number of different fields (Amigo et al. 2015), including grain quality analysis in cereals (Lombi et al. 2011; Caporaso et al. 2018).
Finally, all spectroscopy based technologies need the creation of a calibration model to quantitatively and qualitatively relate the measured spectra with the phenotypic values. A number of different approaches exists to establish these models, like principal component regression, partial least squares, and artificial neural networks (Batten 1998; Foley et al. 1998; Cen and He 2007; ElMasry and Sun 2010; Li et al. 2014). Independent of the approach, they all rely on conventional wet lab measurement of a smaller number of calibration samples, which are used to model (or predict) the phenotypic values based on the measured spectra (Batten 1998; Foley et al. 1998; Cen and He 2007; ElMasry and Sun 2010; Li et al. 2014).

I.VII. Digging for new variation

The above mentioned challenges of agriculture and plant breeding like the re-adaptation of our modern crop cultivars to the changed climate conditions rely on the availability of sufficient genetic variation, since variation is the basis for every breeding associated action (Acquaah 2012). Unfortunately, the majority of our crops are characterized by a loss of variation, mainly through domestication and repetitive rounds of selection in the past, which is known as the genetic “bottleneck effect” (Tanksley and McCouch 1997; Zamir 2001). Crop wild relatives are one possible source to replenish the elite gene pool with new or lost variation during selection (Zamir 2001; McCouch et al. 2013; Zhang et al. 2017), especially Vavilov’s centers of origin of crops are rich in diversity (Harlan 1971; Harris 1990), since they harbor numerous wild crop species (Fig. 9, the orange and red spots correspond quite well to Vavilov’s intended centers of origin).

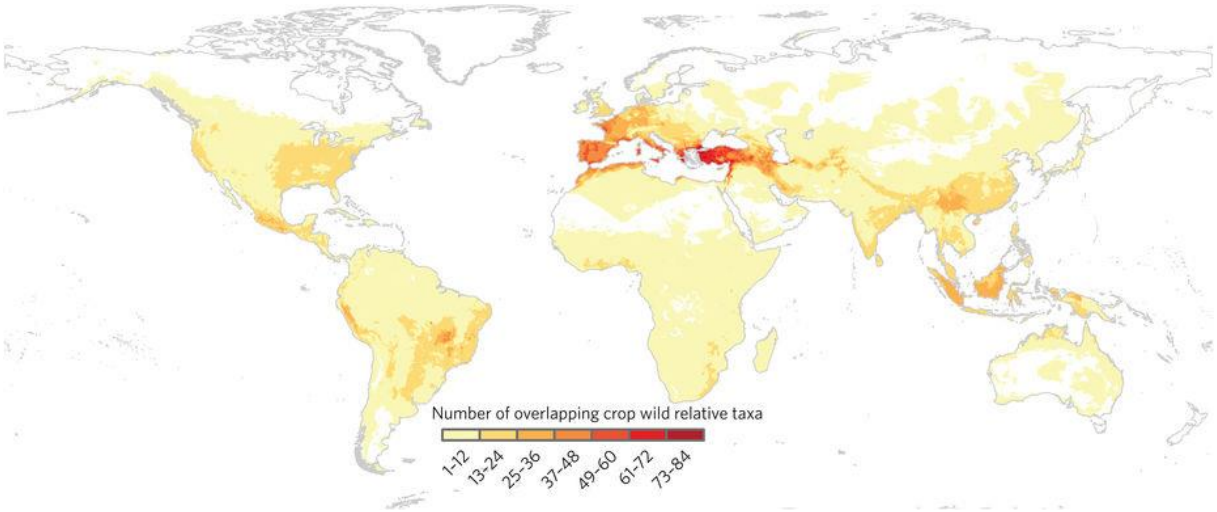

Figure 9 | Crop wild relative taxon richness map

The map displays overlapping potential distribution models for assessed crop wild relatives. Dark red indicates greater overlap of potential distributions of taxa, that is, where greater numbers of crop wild relative taxa occur in the same geographic area (Castañeda-Álvarez et al. 2016).

One of these centers is the Fertile Crescent where the agronomic important *Triticeae* species wheat and barley have been domesticated roughly 10,000 years ago (Badr et al. 2000; Brown et al. 2009). For barley, additional domestication centers are object of discussion, such as Tibet in China as a possible second domestication area (Badr et al. 2000; Morrell and Clegg 2007; Dai et al. 2012).

Crop wild relatives have already been frequently used for the introgression of beneficial alleles into modern gene pools (Zamir 2001; McCouch et al. 2013; Zhang et al. 2017), mainly for resistance against pathogens and tolerance to abiotic stresses (Hajjar and Hodgkin 2007). A good example for this approach is *Hordeum vulgare* ssp. *spontaneum* (wild barley), the progenitor of cultivated barely (Badr et al. 2000). Wild barleys have been examined for disease resistance (Schmalenbach et al. 2008, Vatter et al. 2017), abiotic stress tolerance (Honsdorf et al. 2014; Reuscher et al. 2016, Saade et al. 2016), plant developmental traits (Maurer et al. 2015; Maurer et al. 2016; Nice et al. 2017; Herzig et al. 2018) and quality traits (Korff et al. 2008; Schmalenbach and Pillen 2009; March et al. 2012; Nice et al. 2016). Several of these studies used nested association mapping (NAM) populations as population design (Maurer et al. 2015; Nice et al. 2016), for instance the Halle exotic barley - 25 (HEB-25) population. This experimental population originated from crosses between 25 diverse wild barley accessions with the German elite spring barley cultivar "Barke". The 25 wild barleys originated from Afghanistan, Iran, Iraq, Israel, Lebanon, Syria and Turkey (*Hordeum vulgare* ssp. *spontaneum*), as well as from Tibet in China (*Hordeum vulgare* ssp. *agriocrithon* (Åberg)), reflecting the main centers of barley domestication and a high genetic diversity (for more details see Maurer et al. 2015). NAM

populations offer, in comparison to classical breeding populations (e.g. biparental populations), crucial advantages for the genetic dissection of quantitative traits (Yu et al. 2008; Rakshit et al. 2012) and will be discussed in more detail later.

I.VIII. Genetic mapping

Traditionally the selection of superior genotypes in plant breeding was only based on their phenotypic performance measured in field trials, such as the level of grain yield or resistance against a specific pathogen (Allard 1999; Acquaah 2012). With the advent of molecular markers (DNA markers) in the 1980s a new tool for the identification and selection of superior genotypes emerged in breeding (Bernardo 2008; Bernardo 2010). Marker-assisted selection (MAS) was firstly applied in the animal sector and then quickly adapted by plant geneticists and plant breeders to their needs (Jones et al. 1997; Würschum 2012). In addition, molecular markers are frequently used in molecular biology, for instance for the identification of genes controlling a trait of interest. The basic role of molecular markers is the uncovering of "neutral" variation on the DNA level. In this case neutral means that most molecular markers do not show a visible impact on the phenotype, in comparison to morphological markers (visible markers), since they might be only a single nucleotide difference in a piece of (non-coding) DNA (Jones et al. 1997; Collard et al. 2005).

The first DNA markers developed were restriction fragment length polymorphism (RFLP) markers, which are based on the digestion of DNA through restriction enzymes. Each enzyme cuts at a specific nucleotide sequence, generating different lengths of a DNA sequence, which can be recognized via gel electrophoresis followed by DNA hybridization (Fig. 10a; Jones et al. 1997). Genotypes differ in their number of restriction sites in the DNA and, therefore, can be distinguished from each other (Jones et al. 1997). Until today a number of new marker systems emerged, whereupon the most common are single nucleotide polymorphism (SNP) markers (Ganal and Röder 2007; Jones et al. 2009; Davey et al. 2011). In comparison to RFLP markers they hold fundamental advantages like a higher abundance in the genome, easier detection and lower costs combined with the ability to be analyzed in high-throughput. However, they have one disadvantage as they usually distinguish only two allelic forms (Jones et al. 2009).

Another prerequisite for genetic mapping is the presence of genetic trait variation (Bernardo 2008; Jones et al. 2009). A simple example would be the crossing of a small and tall parent regarding plant height. Ideally, both parents should be completely homozygous. The resulting F_1 generation would be absolutely uniform and heterozygous. To generate the required segregating population, the progeny can be selfed (F_2) or backcrossed (BC_1) to one of the parents (Fig. 10a). This population reflects a classical biparental mapping or breeding population, which is assumed to segregate at each polymorphic locus of the genome in a 1:2:1 ratio in case of an F_2 (homozygous small parent: heterozygous: homozygous tall parent).

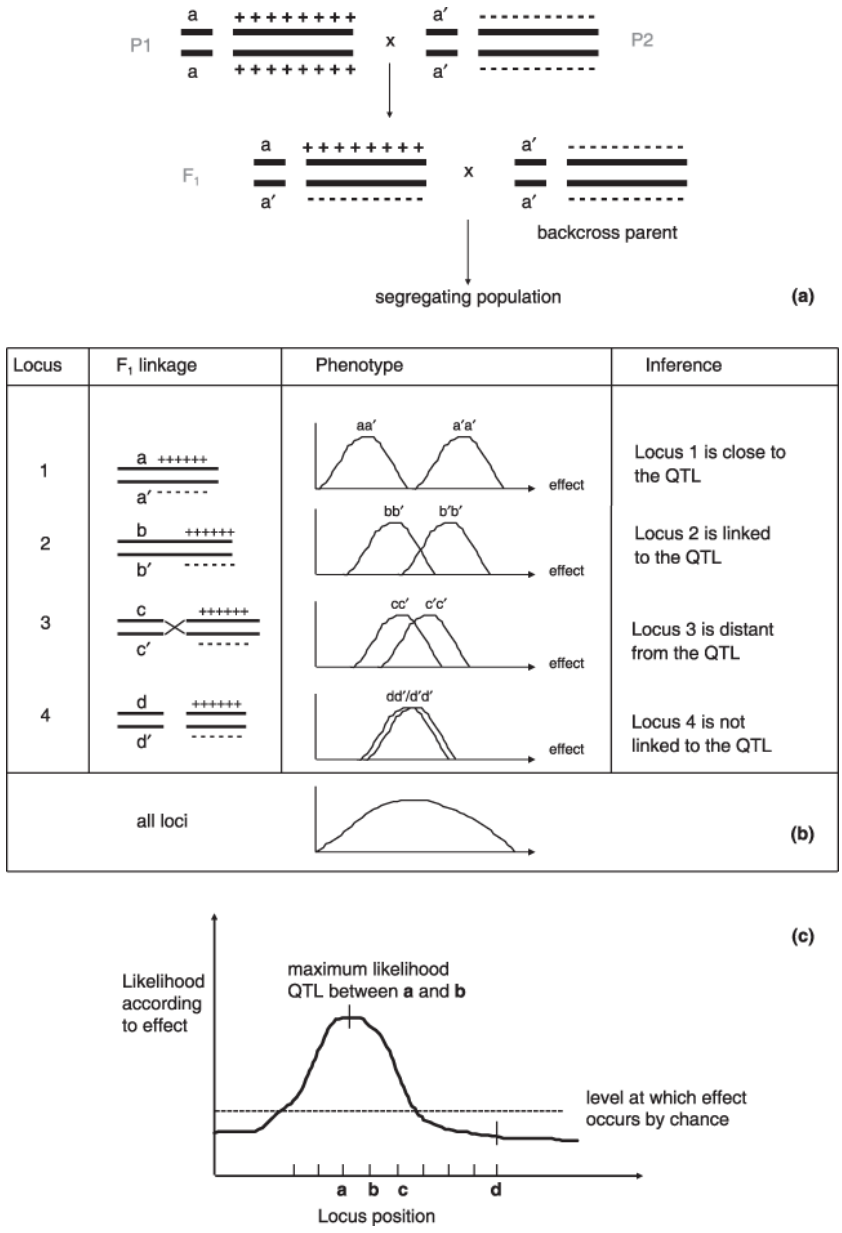

Figure 10 | Composite diagram of the procedure for mapping a quantitative trait locus (QTL)
(a) A mapping population is established by crossing parents, which are divergent for their restriction fragment length polymorphism (RFLP) markers and for the quantitative character concerned (plant height). The heterozygous F_1 is then backcrossed to one of the parents to give the segregating population.

(b) The linkage between the QTL and various marker loci can then be ascertained by the way in which height distribution patterns are associated with the segregation of the two alleles at each locus.
(c) The map position of the QTL is determined as the maximum likelihood from the distribution of likelihood values (ratio of likelihood that the effect occurs by linkage: likelihood that the effect occurs by chance) calculated for each locus (Jones et al. 2009).

For dissecting the genetics of quantitative traits like the above mentioned plant height, the segregating population needs to be phenotyped and genotyped with molecular markers. Subsequently statistical models are applied for the identification of marker trait associations (MTAs) in the genome, which will be finally condensed to quantitative trait loci (QTL) controlling the trait of interest (Fig. 10; Jones et al. 2009; Bernardo 2010). Several strategies for genetic mapping exist, whereupon linkage mapping (also known as family mapping) in segregating biparental populations and association mapping (also known as population mapping) in natural populations or genotype collections are the two classical principles (Jones et al. 2009). Nested association mapping in specifically designed NAM populations represents a combination of both methods (Yu et al. 2008; Rakshit et al. 2012). Independent of the method, they all have the same aim, the identification of genotype phenotype associations (also called MTAs) through detection of polymorphism, which are linked or rather in linkage disequilibrium (LD) to the functional allele(s) (Myles et al. 2009). The simplest approach for the identification of MTAs is the single marker t-test, where the means of two marker alleles are compared by a t-test. A significant p-value would indicate that the respective marker pinpoints to a genomic region affecting the trait of interest (Kearsey and Farquhar 1998; Collard et al. 2005).

There does not exist a perfect mapping strategy, they all have specific pros and cons (Yu et al. 2008; Xu et al. 2017; for details about linkage mapping see Lander and Botstein (1989); for association mapping see Myles et al. (2009); and for nested association mapping see Li et al. (2011)). Nevertheless, there are general factors influencing the success of genetic mapping studies independently of the selected strategy. The most important factors are the genetic properties of a QTL controlling the trait, environmental effects, population size, experimental error and data quality (Collard et al. 2005). Genetic properties comprise the effect size of QTL as well as the space between linked QTL. The detection of QTL is limited if they have minor effects and/or are tightly linked to each other, since they cannot be identified as single loci (Tanksley 1993). A multiplicity of surveys stated the importance of population size for trustworthy QTL mapping results. In smaller populations, genetic mapping is considerably hampered. Firstly, because of a

lower efficiency to detect QTL and secondly, because of an overestimation of their effects, as well as their impact on the explained phenotypic variance (Melchinger et al. 1998; Utz et al. 2000; Collard et al. 2005). These drawbacks can be eliminated by raising the population size and the number of tested environments, whereupon a higher population size is more effective than increasing the number of environments (Schön et al. 2004).

In addition to population size the population design significantly influences genetic mapping, since it defines the mapping strategy as mentioned above. A recent development was the introduction of multi-parental mapping populations like MAGIC (multi-parent advanced generation inter-cross; Cavanagh et al. 2008) and NAM populations (Yu et al. 2008). The use of such populations offers to exploit a higher allele richness and statistical power (Yu et al. 2008; Xu et al. 2017), as well as to circumvent the negative impact of population structure in association mapping of genotype collections (Cavanagh et al. 2008; Myles et al. 2009). Nevertheless, it should be noticed that the creation of these populations takes several years and they are not used in normal breeding programs (Yu et al. 2008; Cavanagh et al. 2008).

The first NAM population in plants was the US maize NAM population developed by Yu et al. (2008). This type of population is distinctively characterized by its unique crossing scheme, where a single common elite line is crossed with multiple exotic donor accessions. The resulting progenies are selfed for several rounds to generate homozygous recombinant inbred line (RIL) families. The number of families corresponds to the number of founder lines used (Yu et al. 2008). Recently two NAM populations have been released in barley by Maurer et al. (2015) and Nice et al. (2016). The first one is the already mentioned HEB-25 population. A subset of 48 selected HEB-25 lines is used as plant material in the present thesis and is referred to as HEB-YIELD. These lines have been selected for an adequate agronomic performance (defined as a satisfactory threshability and no brittle rachis) enabling to conduct yield trials. In addition, they were selected to segregate at the previously defined four major flowering time loci of HEB-25: *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3* (Maurer et al. 2015; Maurer et al. 2016).

I.IX. Objectives

The present dissertation aims to find answers to the four following topics in barley breeding and research. Firstly, to dissect the interaction between plant development,

grain yield and quality under applied agricultural practices. Secondly, to examine the impacts of flowering time or rather plant development on abiotic stress tolerance. Thirdly, the investigation of wild barley as source to replenish the elite barley gene pool with new variation, especially for yield and quality related traits. And finally, to investigate the possibility of replacing wet chemistry analysis by hyperspectral imaging as a tool for grain quality testing.

Therefore, the thesis is split into three parts:

I. **Conducting a worldwide yield trial to reveal the crosstalk between plant development, yield and stress tolerance.**

By testing the wild barley population HEB-YIELD in Dundee, Halle, Al-Karak, Dubai and Adelaide we wanted to capture the diverse agricultural conditions of low and high yielding environments. In addition, the trials have been conducted under control and stress conditions (like nitrogen deficiency, drought and salt stress) to estimate the effects of abiotic stress on plant fitness, as well as to emphasize the importance of adaption for future cropping systems (Ref: Wiegmann et al. 2019a; Chapter II.I.).

II. **Investigating grain quality in two European environments and the influence of plant development and yield on quality.**

Since cereals provide more than just carbohydrates for animal and human consumption, we decided to survey the relationship between grain yield and mineral and protein concentration in mature barley grains from Dundee and Halle. Furthermore, we wanted to estimate the impact of nitrogen fertilization on nutrient accumulation (Ref: Wiegmann et al. 2019c; Chapter II.II.).

III. **Replacing wet chemistry analysis by hyperspectral imaging for grain quality testing.**

In the past, quality parameters have commonly been measured by wet chemistry based methods. However, these are not suited for high-throughput analyses as required in modern breeding programs. Therefore, we investigated hyperspectral imaging under controlled laboratory conditions to predict mineral nutrient concentrations in grains with high accuracy to, ultimately, overcome the constraints of wet chemistry methods (Ref: Wiegmann et al. 2019b; Chapter II.III.).

II. Scientific paper

II.I. A worldwide yield study

"Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues"

By

Mathias Wiegmann, Andreas Maurer, Anh Pham, Timothy J. March, Ayed Al-Abdallat, William T.B. Thomas, Hazel J. Bull, Mohammed Shahid, Jason Eglinton, Michael Baum, Andrew J. Flavell, Mark Tester and Klaus Pillen

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Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues

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Since the dawn of agriculture, crop yield has always been impaired through abiotic stresses. In a field trial across five locations worldwide, we tested three abiotic stresses, nitrogen deficiency, drought and salinity, using HEB-YIELD, a selected subset of the wild barley nested association mapping population HEB-25. We show that barley flowering time genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3* exert pleiotropic effects on plant development and grain yield. Under field conditions, these effects are strongly influenced by environmental cues like day length and temperature. For example, in Al-Karak, Jordan, the day length-sensitive wild barley allele of *Ppd-H1* was associated with an increase of grain yield by up to 30% compared to the insensitive elite barley allele. The observed yield increase is accompanied by pleiotropic effects of *Ppd-H1* resulting in shorter life cycle, extended grain filling period and increased grain size. Our study indicates that the adequate timing of plant development is crucial to maximize yield formation under harsh environmental conditions. We provide evidence that wild barley alleles, introgressed into elite barley cultivars, can be utilized to support grain yield formation. The presented knowledge may be transferred to related crop species like wheat and rice securing the rising global food demand for cereals.

One of the major challenges that mankind faces is the ability to feed the ever-growing population, especially in the face of increased stresses due to climate change and reduced availability of arable land[1,2]. Different climate prediction models indicate severe effects for large parts of Africa, the Arabian Peninsula and Central South America[3,4], where barley (*Hordeum vulgare* ssp. *vulgare*) still has an crucial role as human food[5]. Barley is mainly used for animal feed and for malt production in large parts of the world. It represents the fourth most important cereal crop on a global scale[5,6].

Barley inherently exhibits a higher level of abiotic stress tolerance than other crops[7–9], which offers the possibility to extend its future production to areas suffering from climate change. Furthermore, the relatively simple diploid genetics of barley and the tight relationship between the members of the *Triticeae* tribe facilitate the transfer of knowledge gained from barley research to other major cereals, for instance, bread wheat, durum wheat and rye[10]. Wild barley (*Hordeum vulgare* ssp. *spontaneum*), originating from the Fertile Crescent and from a

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second area some 1,500–3,000 km farther east, was used to domesticate modern elite barley (*Hordeum vulgare* ssp. *vulgare*) more than 10,000 years ago[11–13]. The usefulness of wild germplasm for future breeding has often been emphasized[14–16], mostly as a source to improve biotic resistance and abiotic stress tolerance rather than to directly increase grain yield[17]. Recent studies in wild barley indicate the existence of vast phenological variation for important agronomic traits[18–25]. Wild barely may thus be an appropriate source to replenish the barley gene pool with novel genetic variation. This variation may be valuable to cope with the challenges arising from climate change[26].

Grain yield depends on developmental phases of a plant's life cycle[27]. In this regard, flowering time is a key event as plants shift from vegetative to reproductive growth, moving towards providing the harvestable yield[28–30]. The optimal timing of this event is crucial as it should occur in the absence of adverse effects like abiotic stresses[31] but also ensuring completion of yield accumulation without encountering further adverse effects in most growing seasons. Therefore, the targeted timing of this phase provides one approach to improve stress tolerance, through stress avoidance, and thus to increase grain yield[32]. Flowering time is mainly controlled by environmental cues like day length (photoperiod) and temperature (especially the exposure to cold temperatures, also termed vernalization)[33–35]. Flowering time is highly heritable and, so far, several major genes controlling flowering time have been discovered in model species and in crop plants[36]. Generally, flowering time genes are classified into at least three families: [I] photoperiod genes (e.g. *Ppd-H1*)[37], [II] vernalization genes (e.g. *Vrn-H1*, *Vrn-H2* and *Vrn-H3*)[33,38] and [III] earliness *per se* (*eps*) genes, the last controlling flowering independently from photoperiod and temperature (e.g. *Sdw1*)[39,40].

Here, we present data of a large field study with the HEB-YIELD population, a selected subset of the wild barley nested association mapping (NAM) population HEB-25[18]. The aim of the study was to examine the interplay between flowering time, stress tolerance and yield. For this purpose, HEB-YIELD was studied at five locations worldwide and during two years under locally relevant abiotic stress conditions. We investigated the role of known flowering time genes on developmental and yield-related traits, as well as how they account for yield and stress tolerance.

Results and Discussion

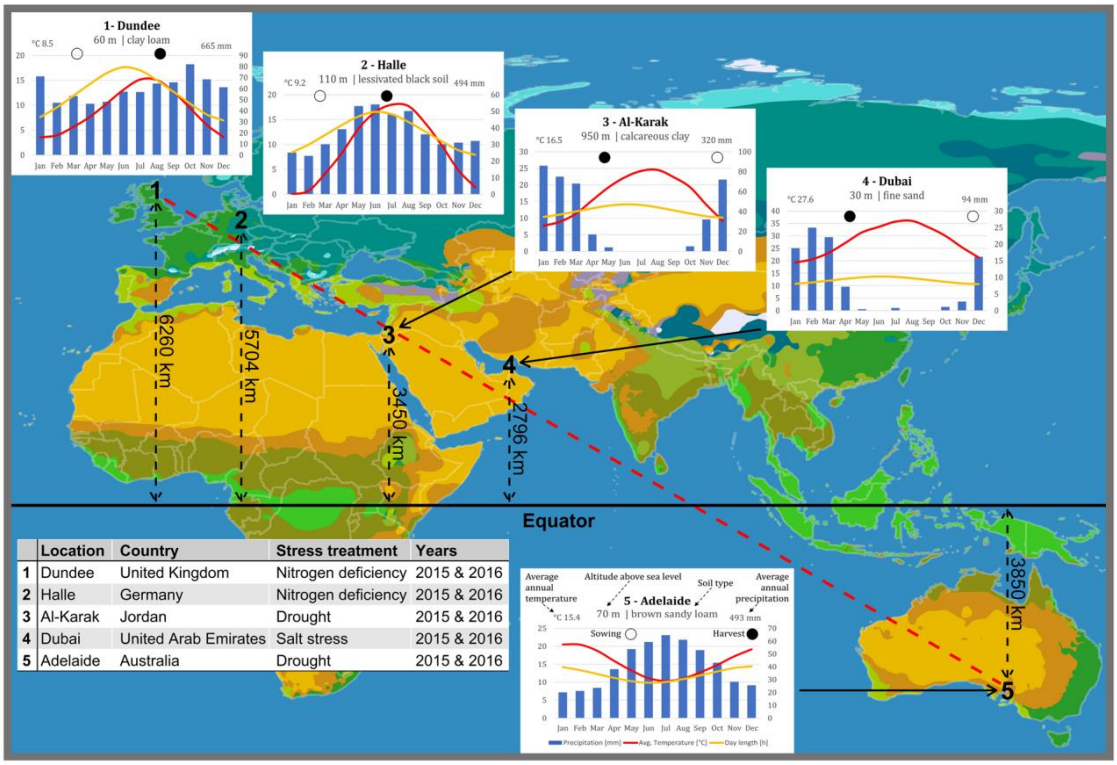
HEB-YIELD exhibits strong phenotypic variation as well as environmental and treatment variation. The wild barley introgression population HEB-YIELD comprises a diverse subset of lines selected from the NAM population HEB-25[18] (Supplementary Table S1a). We studied eleven agronomically traits in a HEB-YIELD trial conducted in Dundee, Halle, Al-Karak, Dubai and Adelaide (Fig. 1; Supplementary Table S2a), where climate data for day length, temperature and precipitation varied considerably between locations Supplementary Figs S1 and S2). The parameters studied included developmental and yield-related traits, used to capture growth variation among HEB-YIELD lines (Supplementary Table S3). At each location, the traits were measured under site-specific abiotic stress conditions, i.e. nitrogen deficiency in Dundee and Halle, drought stress in Al-Karak and Adelaide and salt stress in Dubai. In total, 3,207 field plots were evaluated over all sites, seasons, treatments, and replicates (Supplementary Tables S2c and S4a). Considerable phenotypic variation within locations and treatments was observed for all investigated traits (Fig. 2; Supplementary Table S5).

The ANOVA revealed that all investigated factors (genotype, year and location) were significant for all traits except plant height (HEI) where the year effect was not significant (Supplementary Table S6a). Interestingly, only the traits shoot elongation phase (SEL) and HEI showed comparable values across locations, whereas for the majority of traits pronounced location effects were observed (Supplementary Table S6c). For instance, flowering time varied from 57 to 144 days and grain yield from 0.14 dt/ha to 74 dt/ha (Supplementary Table S5), reflecting a strong diversity in yield potential among the trial sites (Fig. 2).

Irrespective of the diverging agricultural practices at the trial sites, developmental trait heritabilities were high with an average of 0.87, ranging from 0.10 (ripening phase (RIP) under control treatment in Al-Karak) to 0.99 (shooting (SHO) under control treatment in Adelaide as well as flowering (HEA) and SEL under both treatments in Dubai, Supplementary Table S5). In general, yield-related traits revealed lower heritabilities with an average of 0.65. The most complex trait, grain yield (YLD), revealed average heritabilities of 0.73, ranging from 0.05 (YLD under stress treatment in Dubai) to 0.93 (YLD under control treatment in Dundee).

Trait performance in HEB-YIELD is usually a linear transformation from control to stress treatments indicating low genotype by treatment interaction. To gain insights into how abiotic stresses may affect plant development and grain yield, we cultivated HEB-YIELD under contrasting stress conditions, which are relevant for the respective test locations (Supplementary Table S2c). The applied stresses exhibited only minor effects on plant development traits except for HEI. In contrast, strong effects on all measured yield-related traits were observed at all test locations, for instance, reducing yield under stress between 16% in Halle and 65% in Adelaide (Fig. 2; Supplementary Table S5). We observed a weak trend, that HEB-YIELD lines under drought and salt stress exhibited an accelerated plant development, presumably to escape the stress condition, which is in agreement with other studies in cereals[32,41]. Based on our findings we suggest that plant development in the wild barley population HEB-YIELD is mainly determined by genetic factors and to a lesser extend modified by abiotic stresses. This is further supported by the observation that plant developmental traits showed a nearly linear shift between control and stress conditions, as indicated by high correlation coefficients ($0.99 > r > 0.59$) between stress and control treatments of developmental traits, except for SEL in Adelaide ($r = 0.12$; Table 1; Supplementary Table S7a).

Grain yield correlations indicate that yield formation depends on a location-specific interplay between developmental traits and yield components. We observed Pearson correlations coefficients between plant developmental stages shooting, flowering and maturity ranging from $r = 0.67$ to $r = 0.96$ (apart



	Location	Country	Stress treatment	Years
1	Dundee	United Kingdom	Nitrogen deficiency	2015 & 2016
2	Halle	Germany	Nitrogen deficiency	2015 & 2016
3	Al-Karak	Jordan	Drought	2015 & 2016
4	Dubai	United Arab Emirates	Salt stress	2015 & 2016
5	Adelaide	Australia	Drought	2015 & 2016

Figure 1. Global macroclimate map with information on the five experimental locations. The position of the five (1–5) test locations are indicated on a simplified map of the Köppen-Geiger climate classification system provided by LordToran *"Clickable world map with climate classification"*, https://en.wikipedia.org/wiki/World_map#/media/File:K%C3%B6ppen-vereinfacht.svg, copyright: CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0/). General information about the test locations are given in the table on the lower left-hand side including the nearest town, country, stress treatment and the years of field trials. Insets next to map positions depicts long-term climate information for each test location. The average monthly precipitation in millimeters (blue bars), the average monthly temperature in degrees Celsius (red line) and the course of the day length during the year in hours (yellow line) are displayed. In addition, the sowing and harvesting dates are indicated with empty and filled circles, respectively. The Adelaide inset on the right-hand side serves as a legend for the insets.

from shooting correlations in Dubai Supplementary Tables S6b and S7b), indicating a nearly colinear regulation of plant developmental phases. Thus, HEB-YIELD lines early or late in shooting have the tendency to stay early or late respectively until maturity. This observation is in agreement with previous findings in the wild barley NAM population HEB-25, studied in Halle[20] and Dundee[25]. Consequently, early developmental stages may be used as an indirect criterion to select HEB-YIELD lines for early or late maturity.

Following these findings, we explored the relationship between plant development and yield formation in HEB-YIELD (Table 2). We observed a trend that late plant development is beneficial for increased grain yield under Dundee, Halle and Adelaide growth conditions, indicated by positive correlation coefficients of r(HEAxYLD) = 0.59/0.66, 0.32/0.20 and 0.57/0.51, respectively, under control/stress treatments. This trend fits the general observation that late lines have the potential to exploit a prolonged growing season if the environmental conditions including temperature and precipitation are beneficial[29,42,43]. In contrast, under the harsh environmental conditions at Al-Karak and Dubai, HEB-YIELD lines with accelerated plant development were favored. Consequently, we observed negative correlations between flowering and grain yield at Al-Karak and Dubai with r(HEAxYLD) = −0.30/−0.72 and −0.51/−0.44, respectively, under control/stress treatments (Table 2). Here, elevated temperatures and low rainfall restricted plant growth to a few months and thus earliness is a major breeding goal[44,45]. In future, this situation may intensify, since climate change is expected to further shorten the growing period in drought and heat prone locations like in Jordan[26,32,46].

We also observed strong location-specific correlations between flowering time and yield components. For example, in Halle and Dundee, flowering time was positively correlated with grain number per ear (GNE), with r(HEAxGNE) = 0.67/0.67 and 0.71/0.64, respectively (Table 2). Here the extended vegetative growth phase allowed more spikelet primordia to be maintained. In contrast, in Al-Karak flowering time negatively affected thousand grain weight (TGW) with r(HEAxTGW) = −0.56/−0.68, reflecting a grain filling penalty for later

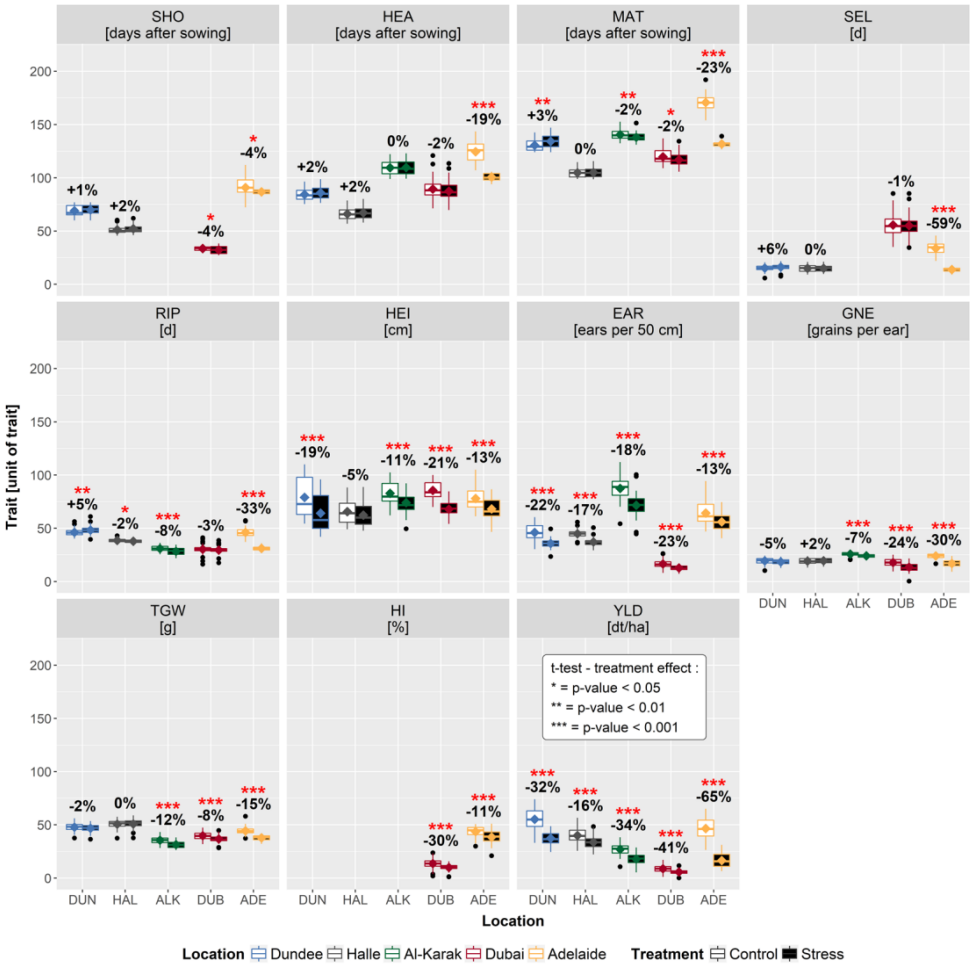


Figure 2. Box-Whisker plots illustrating HEB-YIELD trait variation per location and treatment. Trait names and trait units are indicated in the grey rectangle above each subplot. Trait abbreviations are listed in Supplementary Table S3. The locations Dundee (DUN), Halle (HAL), Al-Karak (ALK), Dubai (DUB) and Adelaide (ADE) are indicated with blue, grey, green, red and yellow box-whiskers, respectively, and, in addition, at the bottom of the plot. Empty and filled boxes refer to control and stress treatments, respectively. Significant differences between treatments are indicated with red asterisks above boxes with $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. The relative increase/decrease (in %) of the stress treatment compared to the control treatment is given below the asterisks.

flowering genotypes. These findings suggest that flowering time controls final grain yield to a certain degree. By comparing correlations between grain yield and yield components, we observed that apparently GNE is the key determinant of grain yield in HEB-YIELD – irrespective of location and treatment. This result is in agreement with earlier studies[47,48], indicating that any increase in number of grains may also improve grain yield[49,50]. We thus reason that improving GNE may offer the best route to increase grain yield in HEB-YIELD independent of the environmental conditions.

The highest positive correlations of yield were found with harvest index (HI; scored only in Dubai and Adelaide with r(YLDxHI) = 0.87 and 0.83, respectively, Table 2). A previous study noted the importance of increasing harvest index to improve yield during the past century[51]. However, a further improvement of grain yield through raising harvest index may be a dead end, since barley is supposed to have reached an optimum with a harvest index of approximately 0.62[51,52]. Therefore, future grain yield improvements may be achieved through increasing plant biomass[51,53]. This suggestion is in accordance with our finding that grain yield exhibited a slightly positive correlation with shoot elongation phase in those environments where lateness was beneficial to increase yield (Table 2). During shoot elongation, which captures the growth period between establishing awn primordia and ear emergence, the leaf growth rate and the potential grain number per area are defined[47,49,54]. An extended shoot elongation phase may thus improve grain yield by increasing leaf size, i.e. biomass, and grain number per area. On the other hand, ripening phase under drought stress exhibited positive and negative correlations with grain yield in Al-Karak and in Adelaide, respectively. Whereas the Adelaide finding fits the assumption that early

Location	Dundee	Halle	Al-Karak	Dubai	Adelaide
Trait[a]	Control vs. nitrogen deficiency	Control vs. nitrogen deficiency	Control vs. drought	Control vs. salt	Control vs. drought
SHO	**0.95**	**0.98**	—	0.22	**0.60**
HEA	**0.99**	**0.99**	**0.96**	**0.98**	**0.87**
MAT	**0.88**	**0.99**	**0.92**	**0.96**	**0.71**
SEL	**0.82**	**0.97**	—	**0.93**	0.12
RIP	**0.70**	**0.94**	**0.86**	**0.89**	**0.59**
HEI	**0.98**	**0.98**	**0.92**	**0.58**	**0.77**
EAR	**0.48**	**0.76**	**0.63**	**0.46**	**0.36**
GNE	**0.89**	**0.97**	**0.61**	**0.35**	**0.44**
TGW	**0.95**	**0.98**	**0.87**	**0.58**	**0.76**
HI	—	—	—	**0.40**	**0.68**
YLD	**0.88**	**0.93**	**0.77**	0.24	**0.80**

Table 1. Location-specific Pearson correlation coefficients (r) within trait, measured under control versus stress condition. Bold values indicate significant correlations at $P < 0.05$. [a]Trait abbreviations are given in Supplementary Table S3; — = trait not scored.

Location	Dundee		Halle		Al-Karak		Dubai		Adelaide	
	+N fert.	−N fert.	+N fert.	−N fert.	+Irriga.	−Irriga.	−Salt	+Salt	−Drought	+Drought
Treatment[a]	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Trait[b]	Flowering		Flowering		Flowering		Flowering		Flowering	
YLD	**0.59**	**0.66**	**0.32**	0.20	**−0.30**	**−0.72**	**−0.51**	**−0.44**	**0.57**	**0.51**
EAR	**−0.38**	−0.05	−0.22	**−0.54**	−0.13	**−0.44**	**−0.52**	**−0.57**	0.25	0.09
GNE	**0.67**	**0.67**	**0.71**	**0.64**	**−0.37**	−0.21	**−0.57**	**−0.58**	0.07	**0.60**
TGW	−0.07	−0.10	−0.08	−0.09	**−0.56**	**−0.68**	**−0.32**	**−0.42**	**0.37**	0.17
HI	—	—	—	—	—	—	**−0.67**	**−0.47**	**0.47**	**0.56**
Trait[b]	Grain yield		Grain yield		Grain yield		Grain yield		Grain yield	
HEA	**0.59**	**0.66**	**0.32**	0.20	**−0.30**	**−0.72**	**−0.51**	**−0.44**	**0.57**	**0.51**
SEL	0.05	0.24	**0.40**	**0.40**	—	—	**−0.55**	**−0.52**	0.16	0.29
RIP	0.10	0.15	−0.16	−0.01	0.13	**0.60**	**0.36**	**0.34**	**−0.29**	**−0.45**
HEI	**−0.56**	**−0.33**	−0.05	−0.01	0.00	0.22	0.25	**0.71**	**−0.69**	**−0.63**
EAR	0.17	0.21	0.09	0.12	**0.54**	**0.47**	**0.52**	**0.32**	**0.42**	**0.35**
GNE	**0.59**	**0.62**	**0.62**	**0.58**	**0.53**	**0.47**	**0.72**	**0.34**	**0.46**	**0.68**
TGW	−0.12	−0.08	0.23	0.19	0.16	**0.53**	0.24	−0.07	−0.01	−0.09
HI	—	—	—	—	—	—	**0.87**	**0.59**	**0.75**	**0.83**

Table 2. Location and treatment specific Pearson correlation coefficients (r) between plant developmental traits and flowering time (upper part) and grain yield (lower part), respectively. Bold values indicate significant correlations at $P < 0.05$. [a]+ = with & − = without; N fert. = nitrogen fertilizer, Irriga. = drip irrigation & Salt = drip irrigation saline water. [b]Trait abbreviations are given in Supplementary Table S3; — = trait not scored.

maturity and thus a short ripening phase may improve grain yield under terminal drought, the Al-Karak finding is unexpected. Under drought stress conditions in Al-Karak, an extended ripening phase was associated with an increase in grain weight, ultimately resulting in elevated grain yields. We conclude that fine-tuning of plant development, especially their sub-phases, may contribute to a better adaptation of improved varieties to their target environment. The latter notion is supported by the finding that in the first instance climate change is expected to impair flowering time[32], which is crucial for plant adaptation and yield formation[49,55]. In addition, our stress treatments confirmed the known association between inflorescence development and stress tolerance/avoidance[32,56]. This offers the possibility to use the genetically relatively well-understood trait flowering time as a proxy to select for improved grain yield under abiotic stresses[57].

Flowering time genes exhibit pleiotropic effects on yield formation in HEB-YIELD. In order to explore the interplay between flowering time regulation and yield formation, we investigated the effects of four major flowering time genes, *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*, in HEB-YIELD (Supplementary Table S8a). The relevance of these candidate genes has been reported in various studies[34,36], including the wild barley NAM population HEB-25[18,20,22,25]. The wild barley lines of HEB-YIELD were selected to compare the effects of wild and

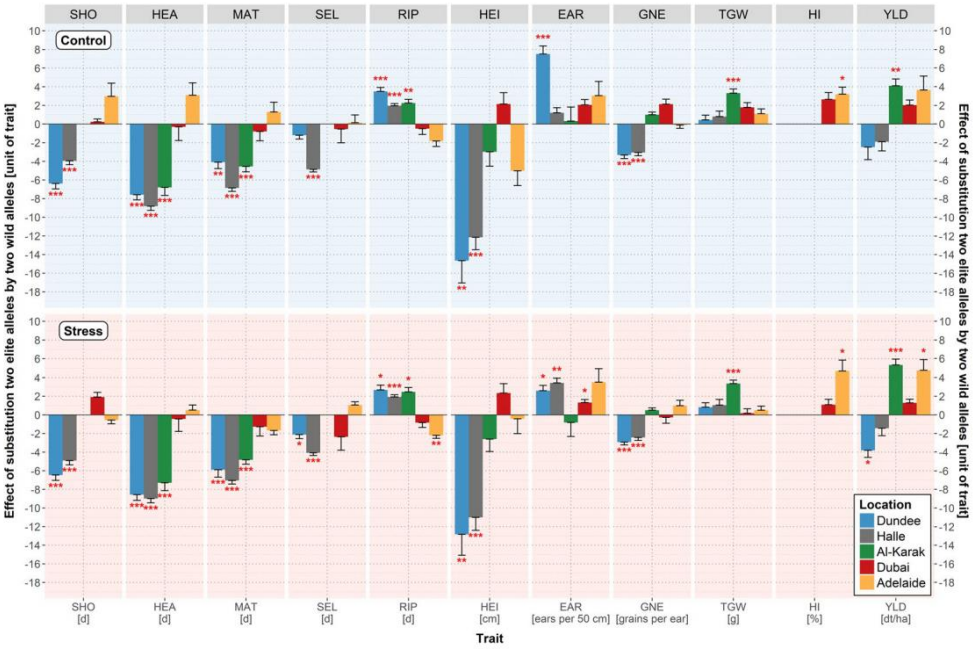


Figure 3. Estimates of *Ppd-H1* wild allele effects on plant developmental and yield-related traits. The trait names are given in the grey rectangles above each subplot and at the bottom where, in addition, the units of the traits are indicated. Trait abbreviations are listed in Supplementary Table S3. The color of the bars represents the location, blue for Dundee, grey for Halle, green for Al-Karak, red for Dubai and yellow for Adelaide. *Ppd-H1* wild allele effects under control and stress treatments are depicted with a bright blue (top) and a bright red background (bottom), respectively. Statistically significant wild allele effects are indicated by red asterisks above or below the bars with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The height of the bars indicates the size of the *Ppd-H1* wild allele effect, obtained by calculating the difference between the mean performances of HEB-YIELD lines carrying two wild alleles versus two elite alleles.

cultivated alleles at these four flowering time loci (Supplementary Table S1a). In the following, we report on the pleiotropic effects associated with the four flowering genes studied.

Ppd-H1. Flowering under long days is promoted by the photoperiod responsive *Ppd-H1* (*PHOTOPERIOD-H1*) allele, an orthologue of the Arabidopsis pseudoresponse regulator gene *PRR7*, which is present in wild barley and winter barley cultivars[37]. In contrast, spring barley cultivars like Barke possess the recessive non-responsive *ppd-H1* allele, resulting in late flowering. Allelic variation in *Ppd-H1* follows a latitudinal cline, where a large proportion of the dominant, long day responsive allele is present in cultivars from Southern Europe and the recessive, non-responsive allele, predominates in Northern Europe[58]. Selection of the non-responsive alleles found in elite spring barley allowed barley cultivation in northern latitudes, under more humid and colder conditions[59]. During early plant development, the photoperiod signal is transmitted from the circadian clock oscillator *Ppd-H1* through mediation of the CONSTANS (CO) protein to the floral inducer *Vrn-H3*, an orthologue of the Arabidopsis *FLOWERING LOCUS T (FT)* gene.

In order to locate sequence variants in *Ppd-H1* discriminating between wild and cultivated barley in the set of HEB-YIELD parents, we used exome capture sequence data (Supplementary Table S1b). The data set comprised nine spring barley cultivars (including Barke), six winter barley cultivars and 25 wild barley accessions *(H. v. ssp. spontaneum* and *H. v. ssp. agriocrithon)* including 19 wild barley donors present in HEB-YIELD. We found 107 sequence variants in *Ppd-H1* (Supplementary Table S1b). Fourteen sequence variants in *Ppd-H1* are notable. Variant 97 at chr2H_29.127.381 bp discriminated between all tested wild and spring barleys. This variant is identical with SNP 22 cited in Turner *et al.*[37]. SNP 22 separates winter and wild barley from spring barley and is located in the CCT domain of *Ppd-H1*, where the G to T substitution causes a glycine to tryptophan change resulting in a photoperiod non-responsive mutant phenotype[37]. In addition, 13 *Ppd-H1* variants (15, 27, 30, 37, 39, 42, 51, 62, 64, 88, 99, 112, 116 46) discriminated between the tested wild barleys and all tested spring barleys except HEB-25 donor HID138, originating from Iran but not present in HEB-YIELD[18]. The variants mentioned are located in three introns and five exons and potentially indicate a different functional response of the *Ppd-H1* allele compared to the donor alleles present in HEB-YIELD.

Among the candidate genes, *Ppd-H1* revealed the most pronounced effects on plant development in Dundee, Halle and Al-Karak (Fig. 3; Supplementary Table S8a). This finding is in accordance with several other studies conducted in barley[20,23,25,60,61]. At these locations, the wild allele of *Ppd-H1* accelerated plant development in HEB-YIELD (SHO, HEA and maturity (MAT)) with a maximum effect of −9.0 days in Halle. In contrast, no

significant effect of the *Ppd-H1* wild allele was observed in Dubai and Adelaide. Most wild barley accessions carry the dominant allele, which is responsive to a long day photoperiod, accelerating plant development through upregulating of *Vrn-H3*/*HvFT1*[62,63]. One possible explanation for contrasting effects between locations is the different day lengths at these sites. Dundee and Halle are more than 5,700 km distant from the equator and are clearly exposed to long day conditions indicated by average day lengths of more than 15 hours during shooting phase, which is necessary to trigger the effect of *Ppd-H1*[37]. In Al-Karak, with a day length of appr. 12 hours during shooting phase, we still observed strong *Ppd-H1* effects, although this location is more than 2,000 km closer to the equator than Halle. In Dubai, where day length is shorter with less than 11 hours during shooting phase, only one minor *Ppd-H1* effect on plant development and yield formation was observed (Supplementary Table S8b). Apparently, this is because *Ppd-H1* is only active under long-day condition[37]. Presumably, the short-day signal to initiate flower development in Dubai is transmitted through *Ppd-H2*, the *FLOWERING LOCUS T3 (FT3)* gene[64]. The dominant functional allele of *Ppd-H2* promotes spikelet formation under short-day in spring barley and winter barley originating from Southern Europe whereas the recessive non-functional allele carries a large deletion in the transcribed coding region, which is typically present in Northern European winter barley cultivars[65–67]. Based on exome capture sequence data, we found nine sequence variants in *Ppd-H2* (Supplementary Table S1b). No sequence variant could be identified, which perfectly discriminated between cultivated and wild barley. However, *Ppd-H2* variant 3 at chr1H_514.098.364 bp and variant 7 at chr1H_514.098.702 bp discriminated between the tested winter barleys and the tested spring barleys except Morex. The wild barley HEB-YIELD donors showed SNPs in common but not restricted to spring or winter alleles.

In future, follow-up field studies with double HIFs (heterogeneous inbred families) may assist to further characterize the interplay between the *Ppd-H1* and *Ppd-H2* photoperiod receptors under short-day and long-day conditions[68]. For this, four HEB-25 lines, HEB_05_044, HEB_08_149, HEB_16_063 or HEB_22_039, which were simultaneously heterozygous at both loci in generation BC1S3, can be chosen based on Maurer *et al.*[18]. Subsequently, four homozygous allele combinations at the two loci can be selected in the available HEB-25 selfing generation BC1S3:11. After seed multiplication, epistatic effects on flowering time and yield formation can be tested in replicated field trials using the resulting four nearly isogenic double HIFs.

In HEB-YIELD, *Ppd-H1* acted in a location-specific manner on yield-related traits. The most pronounced *Ppd-H1* effect was present in Al-Karak where the day length-sensitive wild barley allele was associated with an increase of grain yield by 4.1 dt/ha (+15%) and 5.3 dt/ha (+30%) under control and drought stress conditions, respectively (Fig. 3; Supplementary Table S8a). The yield effect may be explained through pleiotropic effects of the wild barley *Ppd-H1* allele, which shortened the overall growing season, increased the period of grain filling (RIP) and increased grain size (TGW). A tendency of the *Ppd-H1* wild barley allele towards enhanced grain yields was also observed in Dubai and Adelaide, however, only significant in Adelaide under drought stress (+4.8 dt/ha = +29%). Usually, the location-specific effects of *Ppd-H1* on yield-related traits are in agreement with the preferred length of the growing period. At those locations where earliness is beneficial, the responsive wild allele of *Ppd-H1* exerted increasing effects on yield-related traits, for example in Al-Karak, where early plants escaped higher temperatures and terminal drought at the end of the growing season. On the other hand, where lateness is preferable to achieve higher yields, the elite barley *ppd-H1* allele increased yield-related traits, for example, in Dundee and Halle. At those locations late HEB-YIELD lines benefited from the extended growing period since the environmental conditions supported plant growth under suitable conditions.

Sdw1. *Sdw1* belongs to the group of so-called semi-dwarfing genes[40], which are responsible for yield elevations during the 'Green Revolution'[69]. Wild barley accessions possess the functional and dominant *Sdw1* allele, a gibberellic acid 20 oxidase *(GA20ox)* gene, which promotes plant growth. In contrast, the recessive, GA-deficient *sdw1* allele[70,71] is present in barley cultivars like Barke, causing a semi-dwarf phenotype. Several studies have shown that semi-dwarfs exhibit reduced plant height, late maturity, increased tiller numbers and an improved harvest index, ultimately resulting in elevated grain yields[40,72,73]. Based on exome capture sequence data, we found 46 sequence variants in *Sdw1* (Supplementary Table S1b). No *Sdw1* sequence variant could be identified to completely discriminate cultivated and wild barley. However, variant 130 at chr3H_634.078.282 bp discriminated all tested cultivated spring and winter barleys from the tested wild barleys except of the three HEB-25 donors HID003, HID099 and HID114, originating from Iraq, Syria and Lebanon, respectively[18]. It is, thus, possible that new *GA20ox* mutations are present in wild barley.

The reported pleiotropic effects of *Sdw1* are also supported by HEB-YIELD field data (Fig. 4; Supplementary Table S8a). Throughout plant development, we detected an accelerating effect of the wild barley *Sdw1* allele in HEB-YIELD, accelerating grain maturity by 4.0 to 8.9 days in Dundee, Al-Karak, Dubai and Adelaide, compared to the semi-dwarfing allele of Barke. Most striking was the pronounced delay of development in Adelaide under the control condition (precipitation = 484 mm), with up to 13 days for SHO. Whereas under stress (precipitation = 159 mm) the effects were on a similar level as in the other locations. The Adelaide effect might be explained by different environmental cues between the two years, resulting from the earlier sowing date and the prolonged growing period of 50 days in 2016. So far, there is no evidence that day length or precipitation affects the function of *Sdw1*[40,71]. However, a wheat survey under controlled conditions already reported that temperature can modify GA dependent responses, where elevated temperatures increase the abundance of GA[74].

The most prominent effect of semi-dwarfing genes is their control of plant architecture, in particular, plant height[40,75]. We confirmed this effect in HEB-YIELD since the wild barley allele increased plant height at all locations and under both treatments with a maximum increase of 33.2 cm in Dundee under control condition. The dominance of semi-dwarf genes in modern crop cultivars indicates their global importance for agriculture[69,76].

This notion is also confirmed in HEB-YIELD where the Barke semi-dwarf allele was associated with an increase in grain yield (Fig. 4; Supplementary Table S8a). In turn, the wild barley allele significantly reduced grain yield, for instance under control conditions in Dundee, Halle and Adelaide by up to 15.8 dt/ha. Under drought

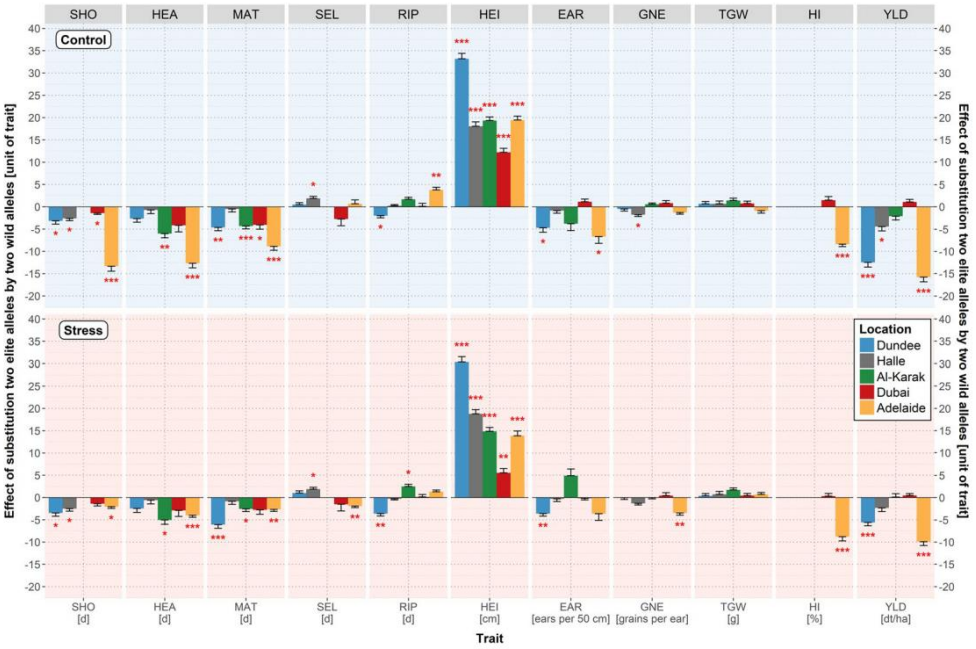


Figure 4. Estimates of *Sdw1* wild allele effects on plant developmental and yield-related traits. The trait names are given in the grey rectangles above each subplot and at the bottom where, in addition, the units of the traits are indicated. Trait abbreviations are listed in Supplementary Table S3. The color of the bars represents the location, blue for Dundee, grey for Halle, green for Al-Karak, red for Dubai and yellow for Adelaide. *Sdw1* wild allele effects under control and stress treatments are depicted with a bright blue (top) and a bright red background (bottom), respectively. Statistically significant wild allele effects are indicated by red asterisks above or below the bars with $*P < 0.05$, $**P < 0.01$ or $***P < 0.001$. The height of the bars indicates the size of the *Sdw1* wild allele effect, obtained by calculating the difference between the mean performances of HEB-YIELD lines carrying two wild alleles versus two elite alleles.

stress conditions in Adelaide, the Barke semi-dwarf allele revealed the strongest impact, accounting for 60% of the final yield level. The observed yield increase may be attributed to an accumulation of several positive effects, including an extended growing period, more tillers, a higher harvest index and less lodging and head loss (Fig. 4; Supplementary Table S8a).

Vrn-H1. In addition to the aforementioned photoperiod and GA dependent pathways, flowering time is also regulated through the vernalization pathway, where exposure to cold temperatures accelerates flowering[33,77,78]. In barley, the response to cold temperatures is mainly controlled by interaction of the two vernalization genes, *Vrn-H1*[79] and *Vrn-H2*[80]. *Vrn-H2* acts as a strong repressor of flowering under long day conditions, preventing winter barley cultivars and wild barley accessions to flower during winter[80]. The expression of the *APETALA1 MADS-box gene Vrn-H1* is only induced after extended periods of cold exposure[81], resulting in down-regulation of *Vrn-H2* and induction of flower initiation through direct binding of the Vrn-H1 protein to the promoters of *Vrn-H2* (repression) and *Vrn-H3* (activation)[82]. In spring barley cultivars like Barke, the dominant *Vrn-H1* allele promotes flowering whereas the recessive winter barley and wild barley alleles delay flowering if cold exposure is imperfect.

Based on exome capture sequence data, we found a huge number of 377 sequence variants in *Vrn-H1* (Supplementary Table S1b). A number of *Vrn-H1* sequence variants discriminated between cultivated and wild barley. For example, variants 475 and 499 at chr5H_599.131.041 bp and chr5H_599.131.479 bp, respectively, discriminated the tested spring barleys from the tested wild and winter barleys. Interestingly, we found one variant, 443 at chr5H_599.130.360 bp, which discriminated the tested spring and winter barleys from the tested wild barleys. In addition, ten *Vrn-H1* variants (187, 194, 289, 296, 335, 340, 388, 452, 457, 498), discriminated the tested winter barleys from the tested spring and wild barleys. The named variants are exclusively located in introns between exon 1 and exon 4. It was already known that deletions in the first intron of *Vrn-H1* result in spring type cultivars, lacking the vernalization need to initiate flowering[82]. Our findings indicate that additional intron regulatory elements may be present in *Vrn-H1* to differentiate winter barley, spring barley and wild barley.

In future, follow-up field studies using nearly isogenic HIFs may assist to characterize developmental and yield formation effects of individual *Vrn-H1* variants, which are present in HEB-YIELD lines[68]. Likewise, the epistatic interaction between selected alleles of *Vrn-H1* and *Vrn-H2* may be characterized in double HIFs, which can be developed from any of five double heterozygous HEB-25 lines, HEB_09_101, HEB_16_095, HEB_16_099, HEB_23_061 or HEB_24_066, as mentioned before.

In HEB-YIELD, *Vrn-H1* exhibited considerable effects on nearly every trait in Dubai and, to a lesser extent, in Adelaide (Supplementary Fig. S3; Supplementary Table S8a). HEB-YIELD lines carrying the wild barley allele at this locus delayed flowering time and maturity by more than 10 days in Dubai. In Adelaide, pronounced effects on plant development were restricted to the control condition (i.e. the Adelaide growing period 2016). Most likely, this effect is caused by warmer temperatures and therefore less vernalization stimuli at the beginning of the growing season (Supplementary Figs S1 and S2; Supplementary Table S9). However, the late development effect of the wild barley *Vrn-H1* allele in Adelaide diminished during cultivation from +12 days at shooting, +6 days at flowering to, finally, +4 days at maturity. This tendency was also present in Halle and Al-Karak, although on a much lower level. In contrast, the late development effect of the wild barley *Vrn-H1* allele remained stable throughout plant cultivation in Dubai. This may be because the temperature in Dubai never reached a vernalization-triggering level. In Dubai, the HEB-YIELD lines possessing a wild barley winter allele at *Vrn-H1* thus responded to the lack of vernalization with a late plant development.

In addition to its developmental effects the wild barley allele of *Vrn-H1* exerted significant reducing effects on all yield components of around 25% in Dubai. Consequently, the final grain yield in Dubai was reduced by 3.2 dt/ha under control conditions, which corresponds to 37% of the total yield. At locations where earliness is the preferred breeding goal and vernalizing conditions are rare, the use of the dominant elite barley allele of *Vrn-H1* is highly recommended.

Vrn-H3. As mentioned before, the expression level of *Vrn-H1* increases with exposure to cold temperatures, resulting in flower induction through repression of *Vrn-H2* and activation of *Vrn-H3*[29,82]. *Vrn-H3* corresponds to the *HvFT1* gene, which is an orthologue of the Arabidopsis *FT* gene, the so called 'florigen'[33,83–85] *Vrn-H3* plays a central role in flower induction integrating photoperiod and vernalization signals[84]. Barley alleles of *Vrn-H3* vary regarding the first intron sequence, promoter sequence and copy number. They are widely distributed over winter and spring growth habits[84–86]. Unfortunately, we could not identify *Vrn-H3* variants in our exome capture sequence data. Presumably, *Vrn-H3* produced no variants since all reads were identified as multi-mappers, located at two or more genomic regions simultaneously, and, hence, were ignored by the variant caller. (M. Bayer, personal comm.).

HEB-YIELD field data validated the role of *Vrn-H3* on plant development throughout the growing period in all locations except from Dubai (Supplementary Fig. S4; Supplementary Table S8a). The wild barley allele of *Vrn-H3* slowed down plant development between 2.2 and 6.6 days. Generally, winter genotypes are characterized by carrying a recessive *Vrn-H3* allele, which displays a reduced expression[85]. Most wild barleys possess a winter type[62] and probably harbor a recessive v*rn-H3* allele, which explains the decelerating developmental effects.

Although *Vrn-H3* plays an important role for plant development, we identified only weak, mostly non-significant, impacts on yield-related traits. Only in Al-Karak, the wild allele showed significant reducing effects on grain number per ears (under both treatments) and on grain yield under drought stress (−3.3 dt/ha).

The best wild barley HEB-YIELD lines match the yield performance of high-yielding local check cultivars. The usefulness of wild accessions, related to crop species has been proposed and demonstrated frequently[14,15,87]. Wild barley accessions, in particular *H. v.* ssp. *spontaneum*, the progenitor of cultivated barley have been used to improve disease resistance[24,88] and abiotic stress tolerance[22,73,89,90], as well as plant developmental traits[18,20,25] and quality traits[88,91,92]. The successful use of wild relatives to increase grain yield of barley has not been reported frequently, some exceptions are available[73,93,94]. This may be because of the negative impacts of linked deleterious wild alleles, a phenomenon generally referred to as 'linkage drag'[95]. The HEB-YIELD lines offer the possibility to estimate potentially positive wild allele effects in an adapted genetic background, since they are embedded through backcrossing into the modern elite barley cultivar Barke. In addition, the elite genetic background enables the direct use of HEB-YIELD lines in barley breeding programs.

Based on our two-year field trials, we identified five high yielding HEB-YIELD lines, which showed acceptable grain yield performance, comparable to the recurrent elite parent Barke, across the tested locations. These HEB-YIELD lines are 01_132, 01_104, 10_184, 10_173 and 05_043 (Supplementary Fig. S5; Supplementary Tables S10a–c). They possessed higher grain yields than Barke in Al-Karak (except HEB_10_184). In addition, HEB-YIELD lines 01_132 and 10_184 surpassed the Barke grain yield in Dundee under both stress and control treatments. Furthermore, we identified HEB-YIELD lines, which reached or surpassed the yield level of locally adapted check cultivars (Supplementary Fig. S5; Supplementary Tables S10a–c). These HEB lines are 10_184 and 01_132 in Dundee (Supplementary Fig. S6), 01_132 and 01_104 in Halle (Supplementary Fig. S7), 05_043 and 10_173 in Al-Karak (Fig. 5), 15_082 and 06_116 in Dubai (Supplementary Fig. S8) and 10_184 & 01_132 in Adelaide (Supplementary Fig. S9). For instance, HEB_01_132 surpassed the grain yield of the established local check cultivar 'Navigator' under stress treatment in Adelaide. In addition, under both treatments it was comparable to 'Compass' and 'La Trobe', which have become the dominant commercial cultivars in South Australia. HEB_01_132 also surpassed the grain yield of the local check '58/1 A' under control treatment in Dubai, indicating that this line may be directly suited for cultivation in the respective environments. Likewise, HEB_05_043 and HEB_10_173 outperformed the check cultivar 'Rum' in Al-Karak under drought stress.

Interestingly, HEB-YIELD lines adapted different yield formation strategies at each test location. Compared to the local check cultivars, HEB-YIELD lines had increased numbers of ears (EAR) at Al-Karak, increased thousand grain weights at Dubai in almost all cases, and increased grain numbers per ear under stress at Dundee and Adelaide in many cases (Supplementary Figs S10–S12). This offers the possibility of achieving future yield improvements following a location-specific adaptation route.

The challenges of climate change demand that cultivars need to re-adapt to changing environmental conditions, for instance shorter growing seasons, higher average temperatures during cultivation and more frequently occurring drought periods[26,46,96]. HEB-YIELD lines exhibited a high phenological variation. For instance,

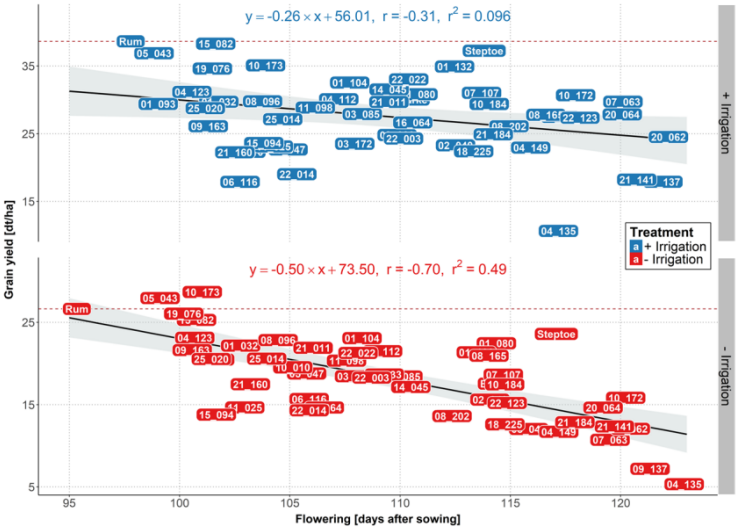


Figure 5. Regression of grain yield on flowering in Al-Karak. The yield levels of the 48 HEB-YIELD lines plus checks are depicted as a function of flowering time, separately for control (blue labels) and stress (red labels) treatments. The yield level of the local check cultivar 'Rum' is indicated by a dashed red line. On top of each subplot the linear regression equation, the Pearson's correlation coefficient (r) and the coefficient of determination (r^2) are indicated.

flowering time exhibited a range of 49 days in Dubai and 36 days in Adelaide (Supplementary Table S5), which offers the potential to use this variation to adapt new cultivars to changing environmental conditions by backcrossing favorable HEB-YIELD donor lines with locally adapted elite cultivars. In those areas where drought and heat affect plant development and grain maturation, early maturing lines like HEB_05_043, HEB_15_082 or HEB_10_173 may be beneficial because of their fast development (Fig. 5; Supplementary Fig. S9; Supplementary Table S10a). Moreover, the increased tiller capacity of HEB-YIELD lines may be promising to achieve an improved canopy cover, reducing moisture losses[26,45] and to increase biomass yield. The latter trait may be high value in the eastern part of the Mediterranean basin where straw and grains of barley are mainly used for animal feeding[97,98].

In the past, Australian varieties followed a strong focus on earliness but changes in agricultural practices have resulted in earlier sowing dates and thus an extended growing season. The earlier sowing allowed later genotypes to benefit from a longer growing period, enabling HEB-YIELD lines HEB_03_085, HEB_10_184, 20_064 and HEB_04_135 to surpass the grain yield of the local check cultivar 'Navigator' in Adelaide under control condition (Supplementary Fig. S9; Supplementary Table S10a). We also identified HEB-YIELD lines that performed quite well in the high yielding environments of Dundee and Halle. Here, lines HEB_01_104, HEB_01_132 and HEB_10_184 accomplished reasonable yields. HEB_10_184, for instance, achieved a maximum grain yield of 74.0 dt/ha under control condition in Dundee, which was almost on par with the local check cultivar 'Odyssey' (−0.3%) and 5.1% higher than the recipient cultivar Barke. Our findings indicate that wild barley HEB-YIELD lines can be used as pre-breeding material to further improve plant development and yield formation of elite barley. (Supplementary Figs S1 and S2; Supplementary Tables S9a and S10a).

Conclusion

It is expected that the impact of climate change necessitates the adaptation of our established crop cultivation systems to harsher environmental conditions[26,96]. Stress avoidance is one promising approach to increase stress tolerance. We explored this relationship by studying the wild barley-derived model population HEB-YIELD in a field experiment, ranging from Dundee in Scotland to Adelaide in South Australia, where the effects of nitrogen deficiency, drought and salinity on plant development and yield-related traits were investigated.

Our findings confirm the crucial relationship between flowering time, plant development and grain yield[99]. The exact timing of the switch from vegetative to reproductive growth under favorable conditions[32], the length of the growing period and the duration of the sub-phases of plant development are crucial to secure yield under abiotic stress conditions. We suggest that adjusting plant development may be a promising breeding strategy to cope with abiotic stresses. To optimize breeding programs, it is thus advisable to first predict the environment-dependent impact of flowering time genes on yield formation and then to select locally advantageous alleles for sustainable crop improvement.

Our HEB-YIELD data indicate that wild germplasm may serve as a resource to increase genetic diversity[14,20,22] and to enable the above mentioned adaptation to abiotic stresses, through selection of early or late development alleles of known major flowering time genes, e.g. *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*. We showed that allelic variants of these flowering time genes strongly react to environmental cues. This information can be used to

design novel breeding strategies such as precise backcrossing of suitable developmental genes into regionally adapted cultivars. Our data also provide evidence that wild barley germplasm may be useful to improve yield in low-yielding environments, for instance, in the Middle East, as well as in high-yielding environments, for instance, in Northern and Central Europe. This knowledge may be transferred to related crop species like wheat and rice to secure the rising global food demand for cereals.

Materials and Methods

Plant material. HEB-YIELD, a subset of the wild barley nested association mapping (NAM) population Halle Exotic Barley-25 (HEB-25[18]), was used in yield trials. HEB-25 originated from crossing 25 diverse wild barley accessions (*Hordeum vulgare* ssp. *spontaneum* and *H.v.* ssp. *agriocrithon*) with the German spring barley elite cultivar Barke (*Hordeum vulgare* ssp. *vulgare*). HEB-25 comprises 1,420 BC_1S_3 derived lines (backcrossed with Barke) grouped into 25 families (for more details see Maurer *et al.*[18]).

The HEB-YIELD subset consists of 48 HEB-25 lines that were selected from HEB-25 to ensure the absence of brittleness and a good threshability enabling accurate yield estimation in field trials. In addition, the final HEB-YIELD lines were selected to independently segregate at four major flowering time loci, which exhibited major plant developmental effects in HEB-25: *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*[18,20,25].

Genotypic data. The complete HEB-25 population was genotyped in generation BC_1S_3 using the barley Infinium iSelect 9k SNP chip (see)[18]. The diagnostic markers i_BK_16, i_12_30924, i_11_10705 and i_12_10218, co-segregating with the four flowering time genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*, respectively, were used for selection of HEB-YIELD lines carrying homozygous elite versus homozygous wild barley alleles. In total, HEB-YIELD includes wild barley chromosomal segments derived from 20 wild barley donors. (Supplementary Table S1a). These donors contributed wild barley alleles at each of the studied four flowering time genes. Supplementary Table S1b provides exome capture-based sequence data of four flowering time genes, *Ppd-H1*, *Ppd-H2*, *sdw1* and *Vrn-H1*, collected through the WHEALBI consortium (https://www.whealbi.eu/) and kindly provided by Drs. Micha Bayer and Joanne Russell, The James Hutton Institute, Dundee, UK.

Field trials. The HEB-YIELD population was grown at five locations worldwide during two years (2015 and 2016), resulting in ten environments. The locations are (from north to south): Dundee (United Kingdom; 56°28′53.71″N 3°6′35.17″W), Halle (Germany; 51°29′46.05″N 11°59′29.58″E), Al-Karak (Jordan; 31°16′34.03″N 35°44′24.94″E), Dubai (United Arab Emirates; 25°5′44.40″N 55°23′24.48″E) and Adelaide (Australia; 35°19′18.5″S 138°53′07.5″E). A detailed description for each location is given in Supplementary Table S2a. The full set of 48 HEB-YIELD lines was cultivated at each location except in Adelaide. Due to lack of seeds, in Adelaide only 34 and 47 HEB-YIELD lines were cultivated in 2015 and 2016, respectively (Supplementary Table S2d). At each location, additional local check cultivars were cultivated, for example: 'Odyssey' (Limagrain, 2011) in Dundee, 'Quench' (Syngenta, 2006) in Halle, 'Rum' (CIMMYT, 1986) in Al-Karak, '58/1 A' (ICBA, 2002) in Dubai and 'Navigator' (University of Adelaide, 2012) in Adelaide.

At each location, a control treatment and a site-specific stress treatment was applied. Stress treatments were nitrogen deficiency in Dundee and Halle, drought stress in Al-Karak, salt stress in Dubai and drought stress in Adelaide (see Supplementary Table S2c). Therefore, lines of the stress treatment received no nitrogen fertilizer in Dundee and Halle, no drip irrigation in Al-Karak and a saline water drip irrigation in Dubai. In Adelaide, only one treatment was applied per season due to lack of seeds. In this case, the two contrasting seasons represented the treatments where 2015 was regarded as the drought stress treatment with only 159 mm precipitation during the growing period and 2016 as the control treatment with 484 mm precipitation.

On average, each HEB-YIELD line was replicated three to four times per treatment. A randomized complete block design was chosen as test design for the trials, with the exception of Dubai and Adelaide where a completely randomized design within each treatment was applied. The trials were conducted in accordance to local practices regarding tillage, fertilization and pest management. Additional information on plant cultivation is provided in Supplementary Table S2b.

Phenotypic data. Eleven developmental and yield related traits were investigated. A description of where and how each trait was measured is given in Supplementary Table S3.

Statistical analyses. All statistical analyses were carried out with SAS 9.4 (SAS Institute Inc., Cary, NC, USA)[100]. Variance components (defined as random) were estimated with *PROC VARCOMP* and broad sense heritabilities (h^2) for each trait within locations and treatments were calculated across years following the formula:

$$h^2_{(control\ or\ stress)} = \frac{V_g}{V_g + \frac{V_{gy}}{Y} + \frac{V_r}{YR}} \quad (1)$$

where

V_g = genotypic variance; Y = number of years

V_{gy} = genotype by year interaction variance; R = number of replications

V_r = error variance

For traits analyzed in a single year, repeatability (rep) was calculated following the formula:

$$rep_{(control\ or\ stress)} = \frac{V_g}{V_g + \frac{V_r}{R}} \quad (2)$$

The analysis of variance (ANOVA) across locations was calculated with *PROC MIXED* to test for the presence of genotype, location and year effects. For this purpose, the main effects (genotype, location and year), as well as their corresponding interaction effects were treated as fixed effects in the following model:

$$Y_{ijk} = \mu + \boldsymbol{g_i} + \boldsymbol{l_j} + \boldsymbol{y_k} + (\boldsymbol{gl})_{\boldsymbol{ij}} + (\boldsymbol{gy})_{\boldsymbol{ik}} + (\boldsymbol{ly})_{\boldsymbol{jk}} + (\boldsymbol{gly})_{\boldsymbol{ijk}} + e_{ijk} \quad (3)$$

where

Y_{ijk} = observed phenotype of the *i*th genotype in the *j*th location and the *k*th year
μ = Intercept
$\boldsymbol{gi}$ = effect of the *i*th genotype
$\boldsymbol{l_j}$ = effect of the *j*th location
$\boldsymbol{y_k}$ = effect of the *k*th year
$(\boldsymbol{gl})_{\boldsymbol{ij}}$ = interaction effect between the *i*th genotype and the *j*th location
$(\boldsymbol{gy})_{\boldsymbol{ik}}$ = interaction effect between the *i*th genotype and the *k*th year
$(\boldsymbol{ly})$ = interaction effect between the *j*th location and the *k*th year
$(\boldsymbol{gly})_{\boldsymbol{ijk}}$ = interaction effect between the *i*th genotype, the *j*th location and the *k*th year
$\boldsymbol{e_{ijk}}$ = residual/error of y_{ijk}

Fixed effects are written in bold

Best linear unbiased estimators (BLUEs) were estimated using the *PROC MIXED* procedure. The BLUEs for each HEB-YIELD line were computed across years and for each treatment level and location separately. Genotype and treatment were modelled as fixed effects and year as a random effect:

$$Y_{ikm} = \mu + \boldsymbol{g_i} + y_k + \mathbf{t_m} + (gy)_{ik} + (\boldsymbol{gt})_{\boldsymbol{im}} + (yt)_{km} + e_{ikm} \quad (4)$$

where

Y_{ikm} = observed phenotype of the *i*th genotype in the *k*th year and the *m*th treatment
μ = Intercept
$\boldsymbol{g_i}$ = effect of the ith genotype
y_k = effect of the *k*th year
$\boldsymbol{t_m}$ = effect of the *m*th treatment
$(gy)_{ik}$ = interaction effect between the *i*th genotype and the *k*th year
$(\boldsymbol{gt})_{\boldsymbol{im}}$ = interaction effect between the *i*th genotype and the *m*th treatment
$(yt)km$ = interaction effect between the *k*th year and the *m*th treatment
e_{ikm} = residual/error of y_{ikm}

Fixed effects are written in bold

For location Adelaide BLUEs were calculated within years and the model was restricted to a fixed effect of genotype.

Pearson correlation coefficients (r) between trait BLUEs were calculated via *PROC CORR*. Furthermore, to test for significant treatment effects a simple t-test (*PROC TTEST*) and an ANOVA within locations were performed (*PROC MIXED*). The ANOVA model included the main effects (genotype, treatment and year) and their corresponding interaction effects as fixed effects (comparable to model III). In addition, a one-factorial ANOVA was computed to test for significant location effects within treatments where only the main effect (location) was included, followed by a Tukey test (*PROC GLM*).

Performance of the HEB-YIELD lines was compared to an adapted check cultivar from the corresponding location (see field trials above) by conducting a Dunnett test[101] (*PROC MIXED*). To enable an easier comparison between the lines the relative performance (RP) was calculated as:

$$(V)\ RP\ [\%] = \frac{(BLUEs\ (HEB\ line) - BLUEs\ (adapted\ check\ cultivar))}{BLUEs\ (adapted\ check\ cultivar)} * 100 \quad (5)$$

To check for significance and estimate effects of the four flowering candidate genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*, a simple linear regression model (*PROC GLM*) was fitted for each candidate gene applying BLUEs across years. Each model included a single locus-specific SNP mentioned above, modeled as a quantitative variable representing the wild allele dosage[20].

All figures were created using R (3.4.2)[102] with the package ggplot2 (2.2.1)[103].

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Author Contributions

M.W. conducted the field trials in 2015 and 2016 in Halle, gathered and analyzed the phenotypic data for all test locations, created the figures, and drafted the manuscript. A.M. planned the field trials in 2015 and 2016 in Halle, supported the analysis of the phenotypic data and drafted the manuscript. A.F., H.B. and W.T. planned and conducted the field trials in 2015 and 2016 in Dundee. M.B. and A.A. planned and conducted the field trials in 2014/15 and 2015/16 in Al-Karak. M.T. and M.S. planned and conducted the field trials in 2014/15 and 2015/16 in Dubai. J.E., A.P. and T.M. planned and conducted the field trials in 2015 and 2016 in Adelaide. K.P. acquired the funding, coordinated the collaboration between the project partners, finalized the exome capture data and drafted the manuscript.

Additional Information

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II.II. Grain quality

„Wild barley serves as a source for biofortification of barley grains"

By

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"Wild barley serves as a source for biofortification of barley grains"

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ABSTRACT

The continuing growth of the human population creates an inevitable necessity for higher crop yields, which are mandatory for the supply with adequate amounts of food. However, increasing grain yield may lead to a reduction of grain quality, such as a decline in protein and mineral nutrient concentrations causing the so-called hidden hunger. To assess the interdependence between quantity and quality and to evaluate the biofortification potential of wild barley, we conducted field studies, examining the interplay between plant development, yield, and nutrient concentrations, using HEB-YIELD, a subset of the wild barley nested association mapping population HEB-25. A huge variation of nutrient concentration in grains was obtained, since we identified lines with a more than 50% higher grain protein, iron, and zinc concentration in comparison to the recurrent parent 'Barke'. We observed a negative relationship between grain yield and nutritional value in barley, indicated by predominantly negative correlations between yield and nutrient concentrations. Analyzing the genetic control of nutrient concentration in mature grains indicated that numerous genomic regions determine the final nutritional value of grains and wild alleles were frequently associated with higher nutrient concentrations. The targeted introgression of wild barley alleles may enable biofortification in future barley breeding.

1. Introduction

Worldwide population growth results in increasing demands for the supply with sufficient amounts of food, as well as superior food quality [1–3]. Cereals, including barley (*Hordeum vulgare* ssp. *vulgare*) as the fourth-most important crop on a global scale [4,5], provide around 50% of the required calories worldwide [3,6]. Their contribution can even account for up to 70% of calories in least developed countries, primarily in Africa and Asia [6], where barley still has a pronounced role as staple food [4,7]. Moreover, cereals function not only as source for carbohydrates, but also for proteins, fiber, and nutrients [8–10], especially in countries where the consumption of animal-based products is unaffordable [3,11]. In addition, over 40% of the world production of barley, maize and wheat is used in livestock feed with the barley proportion in the order of 67% [5,12,13].

The main breeding goal of the 'Green Revolution' was to improve grain yields, which had tremendous success [14,15]. However, the higher yields have one substantial drawback as they lead to a reduction in protein and mineral nutrient contents of grains, reducing their quality and nutritional value [16–18]. Roughly one billion people suffer from low intake of proteins and mineral nutrients, especially iron, zinc, and calcium [19–21]. Furthermore, an adequate supply with nutrients is also necessary for the plant itself to achieve high yields [22]. Therefore, the re-biofortification of our elite crop material represents a worthwhile approach to achieve a balanced diet for humans and livestock [20,23].

Barley represents an appropriate model for cereal research due to its relatively simple diploid genetics [24]. This suits barley as model

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species for members of the *Triticeae* tribe (e.g. soft wheat, durum wheat, and rye), since those species are closely related, allowing to transfer knowledge gained in barley to other *Triticeae* species [24]. Moreover, barley shows high tolerance against abiotic stresses [25–27]. As the already negative impacts of climate change will become more severe in the future, especially in large parts of Africa, the Arabian Peninsula, Southeast Asia and Central South America [28,29], the higher abiotic stress tolerance of barley might be an option to extend its production and provide a secure source for human food.

As a result of domestication and repetitive rounds of selection, many modern crops, including barley, suffer from genetic erosion, which is a loss of genetic variation [30–32]. The introgression of new genetic variation from wild progenitors, like wild barley (*Hordeum vulgare* ssp. *spontaneum*) from the Fertile Crescent and Tibet [33,34], is one option to replenish the gene pools of modern elite crops [31,35]. In this regard a successful example is the *Gpc-B1* locus in bread wheat, which was introgressed from wild emmer (*Triticum turgidum* ssp. *dicoccoides*) into wheat through chromosomal substitution [36,37]. The locus has positive impacts on the concentration of Zn, Fe, Mn and proteins in mature grains without a distinct negative impact on yield [38,39]. Several studies indicated that wild barley harbors huge phenotypic variation for a multitude of agronomic traits [40–45]. However, the usefulness of wild barley as source for biofortification has only rarely been examined.

Therefore, we conducted a study to capture the available variation of macronutrient and micronutrient concentrations in wild barley grains using the wild barley population HEB-YIELD, a selected subset of the nested association mapping (NAM) population HEB-25 [46]. For this purpose, HEB-YIELD was grown during two years in Dundee (United Kingdom) and Halle (Germany) with standard fertilizer application, as well as under nitrogen deficiency to examine the impact of nitrogen supply on mineral nutrient concentrations. In addition, we investigated the interplay between plant development, yield, and mineral concentrations by scoring key agronomical traits throughout the growing season.

2. Material and methods

2.1. Plant material

HEB-YIELD, a subset of the wild barley nested association mapping (NAM) population Halle Exotic Barley-25 (HEB-25) [46], was evaluated in yield trials. HEB-25 originated from crossing 25 diverse wild barley accessions (*Hordeum vulgare* ssp. *spontaneum* and *H. v.* ssp. *agriocrithon*) with the German elite spring barley cultivar Barke (*Hordeum vulgare* ssp. *vulgare*, released in 1996 by breeder Breun). HEB-25 comprises 1420 BC_1S_3-derived lines (backcrossed with Barke) grouped into 25 families (for more details see Maurer et al. [46]).

The HEB-YIELD subset consists of 48 HEB-25 lines that were selected from HEB-25 to ensure good threshability and the absence of brittleness to enable accurate yield estimation in field trials. In addition, the final HEB-YIELD lines were selected to independently segregate for homozygous elite versus homozygous wild barley alleles at the four major flowering time loci, which exhibited major plant developmental effects in HEB-25: *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3* [40,44,46].

2.2. Genotypic data

The complete HEB-25 population was genotyped in generation BC_1S_3 using the barley Infinium iSelect 9k SNP chip (see Maurer et al. [46]). The diagnostic markers i_BK_16, i_12_30924, i_11_10705 and i_12_10218, co-segregating with the four flowering time genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3,* respectively, were used for selection of segregating HEB-YIELD lines that were homozygous for alternative alleles at the four loci (Table S1).

2.3. Field trials

The HEB-YIELD population was grown at two locations during two years (2015 and 2016), resulting in four environments. The locations were Dundee (United Kingdom; 56°28′53.71″N 3°6′35.17″W) and Halle (Germany; 51°29′46.05″N 11°59′29.58″E). A detailed description for each location is given in Table S2a. The full set of the 48 HEB-YIELD lines was sown at both locations (Table S2b). In addition to HEB-YIELD the recurrent parent 'Barke' and local cultivars were used as checks: 'Odyssey' (released by Limagrain, 2011) and 'Tyne' (RAGT, 1986) in Dundee and 'Marthe' (Nordsaat, 2005), 'Quench' (Syngenta, 2006), and 'Scarlett' (Breun, 1995) in Halle.

At both locations the plants were cultivated under regular fertilization (= control condition) and under nitrogen deficiency (= stress condition; Table S2c). In contrast to the control condition, lines in the stress treatment received no additional mineral N fertilizer in Dundee and Halle. The difference between both treatments regarding N were among 60 and 70 kg/N per hectare in both years by considering the results of the N_{min} analysis, which was performed in early spring prior to sowing to determine the availability of N for the HEB-YIELD lines. In Dundee additionally P, K and S were applied to the control blocks following local practice (Table S2c).

A randomized complete block design with four replicates was chosen as test design for the trials (Figure S1). The trials were conducted following local practices regarding tillage and pest management. Additional information on plant cultivation is provided in Table S2d.

2.4. Phenotypic data

In this study 17 traits were investigated and grouped into developmental (e.g. flowering time), yield-related (e.g. grain yield), and grain nutrient traits, including grain raw protein concentration (GPC) and grain concentration of carbon (C), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and sodium (Na). A list of these traits is given in Table S3, including their method of measurement and in which location and year the traits were scored.

2.5. Determination of macronutrients and micronutrients in grains

After air-drying the harvested grains for two weeks, 6–8 g of grains of each plot were ground and homogenized using the mixer mill MM 400 (Retsch GmbH; Haan, Germany).

The dry matter concentration (DM) of each sample was determined after drying the barley flour for 3 h in a drying cabinet at 105 °C (method 3.1 modified [47]).

The elements C, N, and S were measured with a CNS analyzer (vario EL cube; Elementar Analysensysteme, Langenselbold, Germany), which is based on combustion analysis [48]. In this analysis 30 mg of flour per sample were combusted at 1500 °C in oxygen atmosphere for 160 s following the standard protocol of the vario EL cube. During combustion the gaseous products CO_2, NO_2, and SO_2 arose. Subsequently, after transformation and separation, their quantity was measured by a thermal conductivity detector. Grain raw protein concentration (GPC) was calculated by multiplying N by 6.25, according to the general assumption that barley proteins contain on average 16% of nitrogen [49].

For determination of macronutrients (P, K, Ca, Mg), micronutrients (Fe, Mn, Zn, Cu) and Na, inductively coupled plasma - optical emission spectrometry (ICP-OES) was used (Varian 715-ES ICP-OES; Varian, Palo Alto, California, USA). For this, 2 g of flour per sample were combusted in a muffle furnace at 550 °C for 14 h. The resulting ash was digested in three steps by adding two times 10 ml of hydrochloric acid (HCl, 6.0 M) and finally 10 ml of nitric acid (HNO_3, 1.8 M). After addition of HCl the solution was boiled down on a laboratory sand-bath. HNO_3 was evaporated to two thirds of the initial volume. The remaining solution was

transferred into a volumetric flask, filled up to 100 ml with bi-distilled water, filtrated, and analyzed by ICP-OES (methods 10 & 11 modified [47]).

2.6. Statistical analyses

All statistical analyses were carried out with SAS 9.4 (SAS Institute Inc., Cary, NC, USA [50]). Variance components (defined as random) were estimated with *PROC VARCOMP* and broad sense heritabilities (h^2) for each trait within locations and treatments were calculated across years following the formula:

$$h^2_{(control\ or\ stress)} = \frac{V_g}{V_g + \frac{V_{gy}}{Y} + \frac{V_r}{YR}} \quad (1)$$

where

V_g	=	genotypic variance	Y	=	number of years
V_{gy}	=	genotype by year interaction variance	R	=	number of replications
V_r	=	error variance			

In addition, the heritability was calculated within locations but across both treatments:

$$h^2_{(across)} = \frac{V_g}{V_g + \frac{V_{gy}}{Y} + \frac{V_{gt}}{T} + \frac{V_{gyt}}{YT} + \frac{V_r}{YTR}} \quad (2)$$

where

V_{gt}	=	genotype by treatment interaction variance	T	=	number of treatments
V_{gyt}	=	genotype by year by treatment interaction variance			

The repeatability (rep) of each trait was computed for each location and year following the formulas:

$$rep_{(control\ or\ stress)} = \frac{V_g}{V_g + \frac{V_r}{R}} \quad (3)$$

$$rep_{(across)} = \frac{V_g}{V_g + \frac{V_{gt}}{T} + \frac{V_r}{TR}} \quad (4)$$

The analysis of variance (ANOVA) across locations was calculated with *PROC MIXED* to test for the presence of genotype, location and year effects. For this purpose, the main effects (genotype, location and year), as well as their corresponding interaction effects were treated as fixed effects in the following model:

$$y_{ijk} = \mu + \boldsymbol{g_i} + \boldsymbol{l_j} + \boldsymbol{y_k} + (\boldsymbol{gl})_{\boldsymbol{ij}} + (\boldsymbol{gy})_{\boldsymbol{ik}} + (\boldsymbol{ly})_{\boldsymbol{jk}} + (\boldsymbol{gly})_{\boldsymbol{ijk}} + e_{ijk} \quad (5)$$

where

y_{ijk}	=	observed phenotype of the ith genotype in the jth location and the kth year
μ	=	intercept
$\boldsymbol{g_i}$	=	effect of the ith genotype
$\boldsymbol{l_j}$	=	effect of the jth location
$\boldsymbol{y_k}$	=	effect of the kth year
$(\boldsymbol{gl})_{\boldsymbol{ij}}$	=	interaction effect between the ith genotype and the jth location
$(\boldsymbol{gy})_{\boldsymbol{ik}}$	=	interaction effect between the ith genotype and the kth year
$(\boldsymbol{ly})_{\boldsymbol{jk}}$	=	interaction effect between the jth location and the kth year
$(\boldsymbol{gly})_{\boldsymbol{ijk}}$	=	interaction effect between the ith genotype, the jth location and the kth year
e_{ijk}	=	residual/error of y_{ijk}

Fixed effects are written in bold.

Best linear unbiased estimators (BLUEs) were estimated using the *PROC MIXED* procedure. The BLUEs for each HEB-YIELD line were computed across years for each location and treatment level (gt) separately, as well as across treatments (t). Genotype and treatment were modelled as fixed effects and year as a random effect:

$$y_{ikmn} = \mu + \boldsymbol{g_i} + y_k + \mathbf{t_m} + (gy)_{ik} + (\boldsymbol{gt})_{\boldsymbol{im}} + (yt)_{km} + b[yt]_{nkm} + e_{ikmn} \quad (6)$$

where

y_{ikmn}	=	observed phenotype of the ith genotype in the kth year and the nth block in the mth treatment
μ	=	intercept
$\boldsymbol{g_i}$	=	effect of the ith genotype
y_k	=	effect of the kth year
$\boldsymbol{t_m}$	=	effect of the mth treatment
$(gy)_{ik}$	=	interaction effect between the ith genotype and the kth year
$(\boldsymbol{gt})_{\boldsymbol{im}}$	=	interaction effect between the ith genotype and the mth treatment
$(yt)_{km}$	=	interaction effect between the kth year and the mth treatment
$b[yt]_{nmk}$	=	effect of the nth block nested in the kth year and the mth treatment
e_{ikmn}	=	residual/error of y_{ikmn}

Fixed effects are written in bold.

Pearson correlation coefficients (r) between trait BLUEs were calculated via *PROC CORR*. Furthermore, to test for significant treatment effects a *t*-test (*PROC TTEST*) and an ANOVA within locations were performed (*PROC MIXED*). The ANOVA model included the main effects (genotype, treatment and year) and their corresponding interaction effects as fixed effects (comparable to model 5). A further *t*-test was computed to test for differences between the locations.

Performance of the HEB-YIELD lines was compared to the recurrent parent 'Barke' by conducting a Dunnett test [51] with *PROC MIXED*. The resulting *P*-values were adjusted following Bonferroni-Holm [52]. To enable a comparison between the traits the relative performance (RP) was calculated as:

$$RP\ [\%] = \frac{(BLUE\ (HEB\ line) - BLUE\ ('Barke'))}{BLUE\ ('Barke')} * 100 \quad (7)$$

All figures were created using R (3.5.0 [53]) with the package ggplot2 (2.2.1 [54]), except the Circos plots [55].

2.7. Single marker regression

A simple linear model was fit to regress a trait's value on the quantitative SNP marker score obtained from the IBD genotype matrix of Maurer et al. [56]. For this purpose *PROC GLM* was used to fit the model:

$$y = \mu + Marker + e \quad (8)$$

where

y	=	observed phenotype
μ	=	intercept
$Marker$	=	effect of SNP marker
e	=	residual/error

Subsequently, single marker *P*-values resulting from an *F*-test (full model versus reduced model without marker effect) were plotted for each trait in a Circos plot and candidate genes were indicated.

3. Results and discussion

3.1. Phenotypic data

We examined the interplay between plant development, yield, and

nutrient concentrations in the wild barley introgression population HEB-YIELD, a diverse subset selected from the NAM population HEB-25 [46], by scoring 17 traits at two test sites in Dundee (United Kingdom) and Halle (Germany) (Table S2a). The examined traits can be grouped into developmental, yield-related, and nutrient traits, including seven macronutrients (C, N, P, K, S, Ca, Mg) and four micronutrients (Fe, Mn, Zn, Cu), as well as Na (Table S3). All traits were determined under a standard nitrogen fertilizer application regime, following local practice (= control condition) and without nitrogen fertilizer (= stress condition; Table S2c), resulting in 1593 analyzed plots (Table S4a).

The majority of traits exhibited a wide range of variation within each location and treatment, indicated by high coefficients of variation (CV; Figures S2 & S3; Table S5). The extremely low CVs for C with less than 0.5% were striking and confirm that the carbon concentration in plants is very constant with values between 45 and 50% [57]. For the remaining elements, the CVs ranged from around 5% for Mg up to more than 20% for Na, whereby macronutrients showed in general lower CVs than micronutrients.

The ANOVA results indicated that all investigated factors (genotype, year, and location) had significant effects on all traits, except for Cu and ears per square meter (EAR) where location was not significant (Table S6a).

For most nutrients (GPC, P, K, S, Ca, Mg, Fe, Mn, Zn, Cu) we observed heritabilities > 0.54 (on average 0.82; Table 1). The above-mentioned lack of variation for C resulted in low heritabilities at both locations (on average 0.15). Moreover, in Dundee Na had low heritabilities with values < 0.25. The developmental and yield-related traits on average exhibited a heritability of 0.78, whereof the trait EAR was lowest with an average of 0.36.

BLUEs across treatments were calculated to obtain a single value per genotype that allowed an easier comparison with already published results (Table S4b). The observed nutrient concentrations fit very well to those presented in the literature (Figure S4; Table S7), except for Na where the studies of Mengesha [58] and Jeroch et al. [59] reported more than fourfold higher values for barley grains. Elemental concentrations varied substantially between genotypes. We identified genotypes which had more than 50% higher concentrations of Na, Fe, or Zn than the recurrent parent Barke (Figure S5). Notably, line HEB_09_163 had 64% and 50% higher GPC than Barke in Dundee and Halle, respectively. Overall, we identified lines with a maximum GPC of 13.5% and 15.9% in Dundee and Halle, respectively (Table S5). Wild barley might therefore offer a good source for the improvement of GPC, as already indicated by a survey with Tibetan wild barley [60].

3.2. Effects of nitrogen fertilization

Nitrogen undisputedly represents the key nutrient for crops, since this element limits yield in nearly every agricultural cropping system, and there is an increasing demand for it as long as the world population grows. The identification of variation and finally the improvement of the nitrogen use efficiency could be one possible solution to keep the required demand within limits [61,62]. This attempt would help to reduce the costs and energy consumption during the production of inorganic N fertilizer, as well as to secure ecosystems from environmental damage through the application of excessive amounts of N fertilizer [63].

Therefore, we conducted nitrogen deficiency field trials at both locations to assess the effects of the N supply of barley plants on yield and on nutrient concentrations in the grains. The outcome of this experiment indicated that nutrient concentrations in the grains were only to a minor degree influenced by the N supply level of plants, and the treatment effects were noticeably lower than those on grain yield (Fig. 1; Table S5). The only exception from this statement are Zn and Na, which showed a substantial increase in concentration under stress condition (without N fertilizer) in Dundee. Interestingly, the treatment effect for Zn is opposite in Halle in comparison to Dundee and we have no coherent explanation for the different behavior of the N treatment for Zn so far. In any case, the high heritability (> 0.8) points to reliable data. The effects found for grain yield with -17.9 dt /ha and -6.3 dt/ha by comparing control versus stress in Dundee and Halle, respectively, circumstantiate that the treatment was effective and that N is crucial for achieving high yields. The strong N effects on yield are, however, only partly reflected in GPC, especially in Dundee, although N is a main constituent of proteins [63]. In a recently published study Guttieri et al. [64] also observed that N fertilization appears to have only a minor impact on different nutrient concentrations.

In the present study the responses of genotypes to the N treatment were similar, indicated by non-significant genotype-by-treatment

Table 1
Descriptive statistics summary for BLUEs.

Trait[a]		Dundee						Halle					
		Control			Stress			Control			Stress		
	Unit[b]	*Mean*	*CV [%]*	h^2	*Mean*	*CV [%]*	h^2	*Mean*	*CV [%]*	h^2	*Mean*	*CV [%]*	h^2
HEA	days	84.3	6.4	0.91	85.9	7.0	0.91	66.1	8.1	0.95	67.2	8.2	0.95
EAR	number/m^2	603.9	15.2	0.47[c]	468.4	11.5	0.33[c]	571.2	8.4	0.17	470.9	10.5	0.46
GNE	number	19.4	15.9	0.94[c]	18.5	13.7	0.89[c]	19.1	14.5	0.85	19.5	12.5	0.85
TGW	g	47.8	7.3	0.82	46.8	6.9	0.79	50.6	8.0	0.92	50.9	8.3	0.92
YLD	dt/ha	55.3	16.7	0.93	37.4	14.8	0.80	40.1	16.8	0.78	33.7	16.2	0.84
C	% DM	45.9	0.4	0.28	45.7	0.4	0.06	46.5	0.4	0.03	46.5	0.3	0.24
GPC	% DM	9.9	12.3	0.91	9.8	10.2	0.85	13.0	9.1	0.80	11.9	10.0	0.89
P	g/kg DM	3.6	7.6	0.91	3.7	7.0	0.88	3.5	7.7	0.83	3.7	7.3	0.87
K	g/kg DM	4.3	7.3	0.87	4.5	5.9	0.84	4.2	9.3	0.85	4.5	8.7	0.89
S	g/kg DM	1.2	7.8	0.65	1.2	6.4	0.76	1.4	7.2	0.83	1.3	7.2	0.81
Ca	g/kg DM	0.3	10.6	0.79	0.3	10.6	0.81	0.4	11.5	0.86	0.4	12.0	0.91
Mg	g/kg DM	1.2	6.3	0.85	1.2	5.8	0.81	1.2	6.1	0.92	1.2	6.3	0.93
Fe	mg/kg DM	29.9	17.5	0.91	29.7	15.2	0.85	35.2	12.5	0.80	31.9	13.5	0.92
Mn	mg/kg DM	8.0	11.4	0.73	7.7	10.0	0.72	11.1	12.2	0.87	10.6	13.2	0.83
Zn	mg/kg DM	19.8	16.2	0.86	23.1	16.0	0.85	26.7	13.3	0.89	25.3	14.4	0.80
Cu	mg/kg DM	5.0	10.5	0.62	5.2	9.2	0.54	5.0	9.5	0.82	5.0	8.0	0.61
Na	mg/kg DM	31.4	20.7	0.25	40.5	17.9	0.23	72.6	29.6	0.80	74.5	27.4	0.78

a) HEA (Flowering time), EAR (Number of ears), GNE (Grain number per ear), TGW (Thousand grain weight), YLD (Grain yield), C (Carbon), GPC (Grain protein concentration), P (Phosphorus), K (Potassium), S (Sulfur), Ca (Calcium), Mg (Magnesium), Fe (Iron), Mn (Manganese), Zn (Zinc), Cu (Copper) & Na (Sodium).
b) DM (dry matter).
c) Repeatability rather than heritability was calculated as only one year of measurements was available.

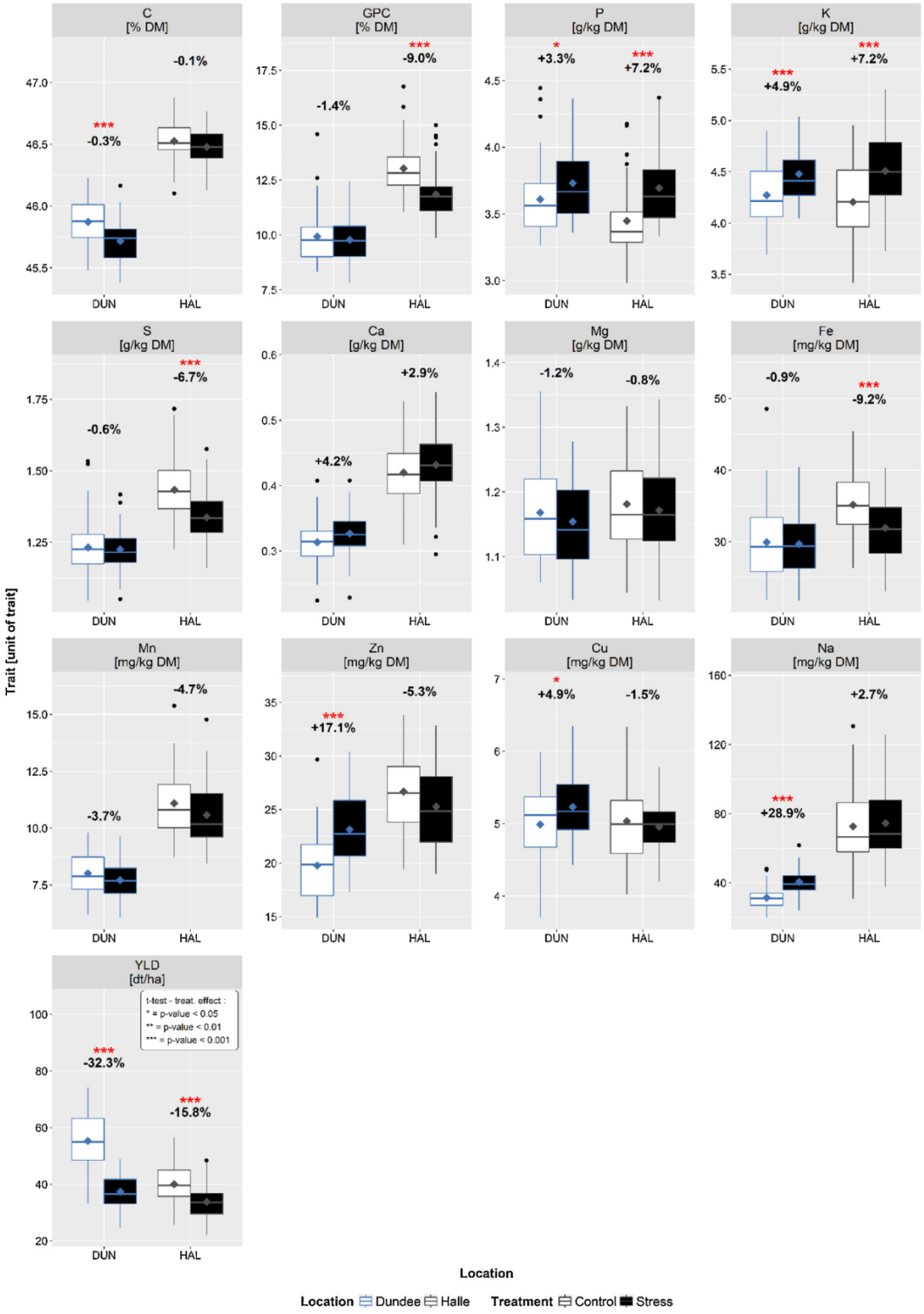

Fig. 1. Trait variation and verification of treatment effects for the studied traits. The trait names and units of the traits are indicated in the grey rectangles above each subplot. Trait abbreviations are listed in Supplementary Table 3. The color of the boxes represents the location, which is also indicated on the x-axis: blue for Dundee (DUN) and grey for Halle (HAL). The y-axis reflects the value of the traits in its specific unit. Non-filled boxes refer to the control condition and filled boxes to the stress condition. Statistically significant treatment effects were obtained via *t*-test and are indicated by red asterisks above the boxes with $P < 0.05$ = *, $P < 0.01$ = ** or $P < 0.001$ = ***. Additionally, the difference between the means of the two treatments is given in relation to the mean of the control condition in percentage above each box. The figure is based on BLUEs across years. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

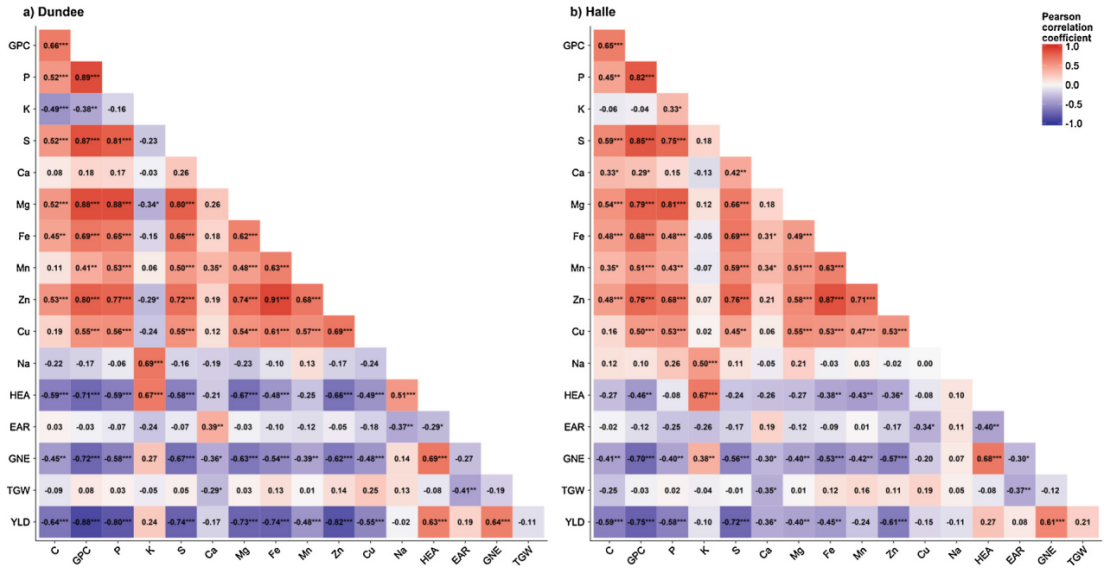

Fig. 2. Pearson correlation heat maps for the studied traits in Dundee (a) and Halle (b). The correlations are colored based on their direction (blue: negative; red: positive) and strength (bright color: weak correlation; dark color: strong correlation). Significance of the correlations is given by asterisks with $P < 0.05$ = *, $P < 0.01$ = ** or $P < 0.001$ = ***. The trait abbreviations are C (Carbon), GPC (Grain protein concentration), P (Phosphorus), K (Potassium), S (Sulfur), Ca (Calcium), Mg (Magnesium), Fe (Iron), Mn (Manganese), Zn (Zinc), Cu (Copper), Na (Sodium), HEA (Flowering), EAR (Number of ears), GNE (Grain number per ear), TGW (Thousand grain weight) and YLD (Grain yield). The figure is based on BLUEs across years and treatments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

interactions for the majority of nutrients (Table S6b). This is also supported by the high positive Pearson's correlation coefficients between control and stress condition for the nutrient traits with an average of 0.84 in Dundee and 0.88 in Halle (Table S8a). Whilst the control plots at Dundee also received supplementary P, K and S, the concentrations of these elements was either less or no different in this treatment when compared to the stress treatment. The same trend was observed at Halle, suggesting that the addition of these elements at Dundee had not biased the results (Table 1). Based on the minor effects of the N deficiency treatment on nutrient concentrations, we decided to merge both datasets for further analyses to increase the statistical power.

3.3. Correlations between plant development, yield, and nutrient traits

To get a first glimpse on the interplay between plant development, yield, and nutrient concentrations, we calculated the correlations between all scored traits across treatments (Fig. 2; Table S8b), as well as within treatments (Figures S6 & S7; Table S8b). Independent of the location, there were two striking findings: first, the high negative correlations between the majority of nutrients (except for K, Ca, and Na) with flowering time (HEA), grain number per ear (GNE), and grain yield (YLD), and second, predominantly positive correlations between most nutrients except K and Na.

The first observation suggests that the mineral concentrations are negatively affected by a 'dilution effect', which is in agreement with a number of previous studies [64–66]. An improvement in yield can be achieved by increasing the grain number per area (= EAR and GNE) or the grain size (= thousand grain weight; TGW) [67,68]. In both cases, the nutrients are distributed into more or larger grains whereas the absolute amount of accumulated nutrients seems to stagnate, causing the well-known 'protein or nitrogen dilution effect' [16,69,70]. In the wild barley introgression population HEB-YIELD, lines that exhibit late HEA and/or high GNE are characterized by superior yields (for more details see Wiegmann et al. [45]), which explains why the nutrient concentrations are also negatively correlated with HEA and GNE. Overall, the negative correlations are more strongly pronounced in Dundee than in Halle, presumably as a result of the higher yield level by 9 dt/ha. Based on the localization of the majority of mineral nutrients in the aleurone layer of a grain, we speculated that high TGW could have a negative impact on nutrient concentrations because larger grains have a reduced surface-to-volume and aleurone to endosperm ratio, resulting in a higher proportion of starch [71]. However, our results indicate no pronounced effects of TGW on nutrient concentrations in general, since the only significant negative correlation of TGW was observed with Ca (-0.29/-0.35; Dundee/Halle), which is in agreement with studies from McDonald et al. [72] and Zhao et al. [66].

The positive correlations between the majority of nutrients indicate that these elements might share common features in uptake, distribution, or storage. Uptake and transport processes are governed by a multiplicity of proteins, which are involved in the steps from mobilization and uptake from the rhizosphere until the final translocation into the seeds. This includes xylem and phloem loading and unloading, tissue distribution, as well as trafficking and sequestration within the cell [73–76]. Examples for the co-handling of mineral nutrients are the transport of both Fe and Mn by transporters of the MTP and NRAMP families [77,78], the transport of Mn and Ca by BICAT proteins [79,80] or the concerted uptake of Fe and Zn through unspecific divalent metal cation transporters [81,82], which is supported by our data as these nutrients showed the highest positive correlations in both locations (0.91/0.87). Recently, a study in barley investigated the role of HvIRT1, a member of the ZIP family of transporters, which is largely responsible for Mn uptake, translocation and accumulation in the mature grain [83]. Moreover, HvIRT1 also transports Zn because of a broad specificity [83]. This interaction is apparent in our data, since concentrations of Mn and Zn showed highly significant positive correlations (0.68/0.71). A further example for the interdependency of nutrient concentrations is the positive relationship between N/GPC and P, possibly due to an increased root growth through N, which improves the P uptake from the rhizosphere [84]. Several independent studies reported the existence of such patterns of correlations between specific nutrients [64,66,85].

In addition, the correlations between K and Na are noteworthy,

since they are mostly contrary to the remaining nutrients. Interestingly, K and Na show only slightly negative or even positive correlations with YLD. The stability of K concentrations in cereal grains under various conditions has been reported before. For example, in a long-term experiment, low K supply rates caused a drop in grain yield of barley and in K concentration in straw [86]. However, K concentrations in grains were invariant and, similar to the findings in the present study, not negatively correlated with yield. This indicates that plants specifically regulate K import during grain filling by unknown mechanisms [87]. K and Na exhibited similar correlations, probably because they partially share the same transport mechanisms and because Na can partly substitute for K in cellular functions [88,89]. Nevertheless, at present it is unclear why K and Na behave different from the majority of other nutrients.

3.4. Improved genotypes for barley breeding

During the last decades wild material has been frequently used for the introgression of genes and alleles encoding for favorable attributes into elite germplasm [31,90]. However, this was mostly successful for the improvement of resistance against pathogens and tolerance to abiotic stresses, rather than the improvement of quality and yield [91]. There have been only a few diversity studies which examined the genetic potential for crop biofortification [64,66,85,92]. The present study is one of the first to evaluate cereal genetic resources to improve grain nutrient concentrations.

The huge phenotypic variation of the “Halle Exotic Barley” wild introgression population has already been exemplified for plant development [40,44], resistance to fungal pathogens [43,93], tolerance to abiotic stresses [41,94] and yield [45,95]. The HEB lines offer the possibility to estimate potentially positive wild allele effects in an adapted background, as they are embedded into the elite barley cultivar Barke (for more details see Maurer et al. [46]). Using such a background enables the direct use of these lines as crossing parents in elite barley breeding programs.

In both locations we could identify HEB-YIELD lines, which significantly outperformed the recurrent parent, cv Barke, regarding the concentration of nearly every investigated nutrient (Fig. 3; Table S9). In particular, for GPC, Ca, Na, Fe, and Zn we could identify a number of promising lines with more than 50% higher elemental concentrations. Some of these lines showed higher concentrations than the recurrent parent Barke for several traits simultaneously, for instance HEB_08_096, HEB_09_163, HEB_11_025, HEB_14_045, HEB_15_082, HEB_15_094, HEB_18_225, HEB_19_076 and HEB_25_020. The increased concentrations in these lines were stable across both investigated environments, although in general nutrient concentrations are influenced by pronounced genotype-by-environment interaction effects (Table S6a) [96,97]. These distinct interactions render it difficult to select superior lines in a breeding program based on a single or few environments [98,99]. Additionally, we found lines that showed increased concentrations of single elements, like for Na (e.g. HEB_07_063 in Dundee and HEB_08_202 in Halle) and Ca (e.g. HEB_10_184 in Dundee and HEB_01_132 in Halle), whereby the latter lines had roughly the same yield as Barke.

Nevertheless, it must be mentioned that the majority of lines had a considerably reduced grain yield. This is best exemplified by HEB_09_163, which exhibited significantly higher concentrations of GPC, P, S, Ca, Mg, Fe, and Zn in both locations, but also a more than 50% lower yield level. As discussed in 3.3, this negative relationship between nutrient concentration and yield is well-described [64–66], making it difficult to select for higher nutrient concentrations without reducing yield. Moreover, a reduction in yield is hardly acceptable, as we need to raise yields in the next decades to supply the growing world population with a sufficient amount of food [2,3].

3.5. Nutrient yield

We further explored the relationship between nutrient concentrations and yield by calculating the nutrient yield (= product of plot grain yield and its respective nutrient concentration; in agreement with Khan et al. [100]).

Nearly all HEB-YIELD lines showed significantly lower nutrient yields than Barke, particularly in Halle where we found strong reductions in nutrient yield for the elements K (up to 55%), Ca (51%), Fe (47%), P (44%) and Zn (44%) (Figure S8; Table S9a). In contrast, in Dundee we could detect lines that had significantly higher nutrient yields for Ca (+32%), Fe (+27%) and Zn (+18%) (Figure S9; Table S9a). However, the general trend is unambiguous that most HEB-YIELD lines had inferior nutrient yields to Barke. There are only a few studies available that investigated the relationship between nutrient concentrations and nutrient yields, but there is an in-depth knowledge present about the relationship between protein concentration and protein yield [16,101–103]. One common observation is that an improvement in protein yield is mainly achieved by raising grain yield rather than protein concentration. This is in agreement with the achievements of the last decades of breeding and selection for higher grain yields, which resulted in lower grain protein concentrations, but improved protein yields [17,102]. Based on our data we suggest that this is also valid for other nutrient yields, since those lines having significantly lower grain yields than Barke are characterized by marginal nutrient yields, whereas the local check cultivars mostly exhibited superior nutrient yields in both locations (for instance GPC: Figure S10, and Zn: Figure S11).

Our findings clearly support the existence of high variations of nutrient concentration in HEB-YIELD and a pronounced negative relationship between yield and the majority of the investigated nutrient concentrations with the exception of K. One breeding approach would be to cross the best-performing HEB-YIELD lines regarding nutrient concentration (e.g. HEB_09_163 for GPC) with a high-yielding elite line and derive a random inbred population that could be used to determine if the two characters can be separated genetically and, if so, identify not only suitable recombinants but also genetic markers that could be used in future selection programs. As reported by Bogard et al. [104] different strategies have already been applied to reduce the negative correlation, including the introgression of genes from related species, even though all of these strategies failed so far.

However, potentially methods based on genetic engineering may be more successful, if they are transferable to field conditions. Two recently published studies report on quite considerable success by overexpressing the Fe and Mn transporter TaVIT2 [105] and the Zn transporter HvMTP1 [106] under control of an endosperm-specific promoter in wheat and barley. In both cases grain nutrient concentrations were improved without negative impacts on growth and yield in greenhouse trials [105,106].

3.6. Associated genomic regions

By dissecting the genetic architecture of the investigated nutrient traits through genetic mapping, quantitative trait loci (QTL) that control the trait variation can be identified [107,108]. Therefore, we applied a single marker regression analysis aiming to identify QTLs that improve nutrient concentration in HEB-YIELD without negative impacts on yield, as recommended by Bogard et al. [109]. However, it must be noted that the relatively small population size of HEB-YIELD (48 lines) might lead to biased results, indicated by a lower QTL detection rate, more false positives, and an overestimation of effect sizes [110–112]. Nevertheless, the obtained results can give a first glimpse on the genetic control of our studied traits. We plan to verify detected QTLs by a follow-up study with heterogeneous inbred families (HIFs [113,114]) segregating for the two alternative alleles present at a promising QTL.

Based on our findings we detected a number of QTLs that

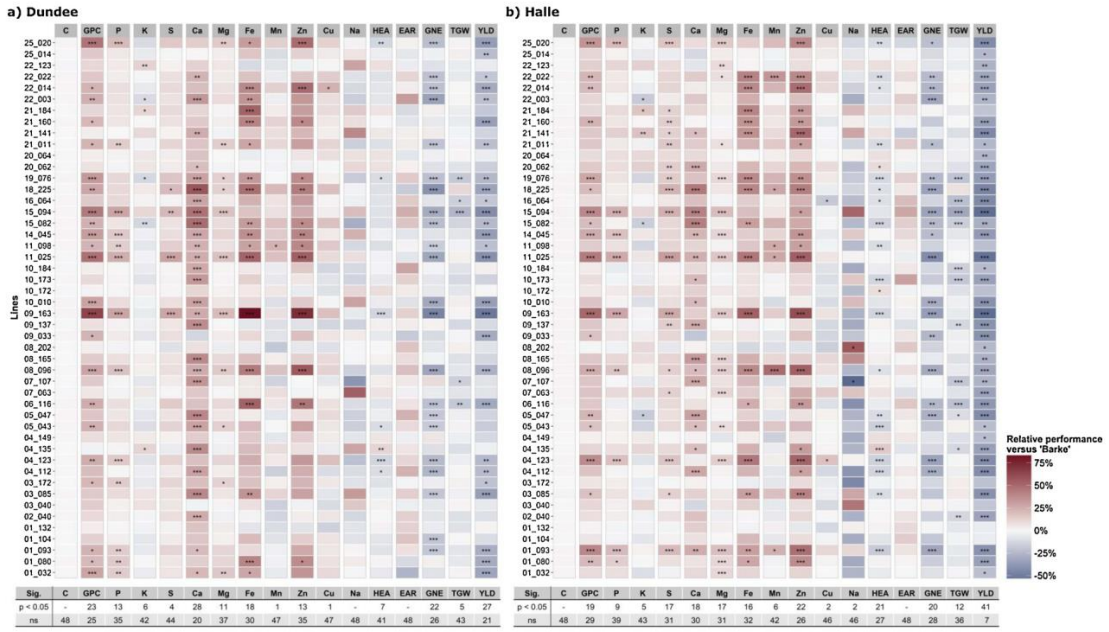

Fig. 3. Heatmap showing the relative performance of the 48 HEB-YIELD lines in comparison to the recurrent parent 'Barke' for the studied traits in Dundee (a) and Halle (b). The color of the tiles represents a positive (red) or negative (blue) deviation from Barke. In addition, the results of a Dunnett's test with Barke as reference are indicated for each line inside the tile. Significant deviations are shown by asterisks with $P < 0.05$ = *, $P < 0.01$ = ** or $P < 0.001$ = ***. The p-values are Bonferroni-Holm corrected, and a summary table of the test is shown below the figure. The trait names are indicated in grey rectangles at the top, and their abbreviations are listed in Supplementary Table 3. The figure is based on BLUEs across years and treatments (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

simultaneously influenced the majority of investigated traits and, in several cases, corresponded to well-known candidate genes (Figs. 4 and 5; Tables S10 & S11). We identified these genomic regions simultaneously in both locations, indicating the robustness of the results. Below we will discuss a few of these regions, indicating the importance of plant development and yield, as well as nutrient uptake and translocation to determine the final grain nutrient concentration in HEB-YIELD.

3.6.1. Short arm of chromosome 2H

We detected significant effects on the traits HEA, K, Fe, Mn, and Zn in both locations, as well as on GPC, Ca, Mg, Cu, and Na in Dundee originating from the short arm of chromosome 2H (Tables S10 & S11). Except for HEA, K, and Na the wild barley allele increased the trait values. In most cases SNP markers, that are located directly within the *Ppd-H1* gene sequence, showed the lowest p-values. HEA effect sizes of around -8 days pinpoint to a possible role of *Ppd-H1*, confirming results already obtained in the whole HEB-25 population [40,44,46]. *Ppd-H1* is the main regulator of photoperiodism in barley and determines flowering time to a high extent [115], as well as exerting pleiotropic effects on a number of additional developmental and yield-related traits [40,44], however without a significant impact on yield in HEB-YIELD [45]. Most wild barley accessions possess the dominant responsive *Ppd-H1* allele, which accelerates development under long-day conditions [116,117]. From studies on Arabidopsis it is known that several nutrient transporters are regulated by the circadian clock and that the expression of PRR7, the *Arabidopsis thaliana* orthologue of *Ppd-H1*, is under clock control [118]. Consequently, the detected effects might be the result of the influence of *Ppd-H1* on nutrient transporter regulation, as well as on overall plant development.

3.6.2. Long arm of chromosome 3H

Sdw1 is the major semi-dwarf gene locus in barley, located on the long arm of chromosome 3H. Exotic *Sdw1* alleles or genes in its proximity exhibited strong effects on our studied traits, especially on plant height [45] and YLD (Tables S10 & S11). In addition, the majority of nutrient traits showed positive effects arising from this region, which clearly supports the negative relationship between yield and nutrient concentration. The region on the long arm of chromosome 3H showed significant effects on GPC, P, Ca, Mg, and Zn in Dundee and Halle, whereupon the wild allele increased all traits except Ca, which was clearly reduced. Semi-dwarf alleles have been widely used in modern breeding programs and were one crucial component of the 'Green Revolution' boosting grain yields in the past [15,119,120]. Semi-dwarf barley cultivars are characterized by reduced plant height, late maturity, increased tiller numbers, and improved harvest index, altogether resulting in elevated grain yields [121,122]. This is in agreement to our observations that HEB-YIELD lines carrying the wild allele (= long straw allele) at *Sdw1* had an increased plant height and a distinctly reduced yield. The reduced yield might be one explanation why most nutrient concentrations showed positive effects coming from the wild allele of *Sdw1*, indicating the important relationship between yield and quality.

3.6.3. Short arm of chromosome 6H

We identified pronounced impacts of the short arm of chromosome 6H on nutrient concentrations, influencing the traits C, GPC, P, S, and Mg at both locations (Tables S10 & S11). In each case, the wild allele increased the nutrient concentration. The senescence-inducing gene *NAM-1*, located on the short arm of 6H [123], might be a probable candidate for this locus. This gene belongs to the family of NAC (NAM, ATAF-1,2, CUC) transcription factors, which influence a multitude of plant processes, such as development and senescence [124]. From studies in wheat and barley it is known that early senescence can improve nutrient concentrations, especially of GPC, Fe, and Zn, accompanied with negative impacts on yield [123,125,126]. These findings

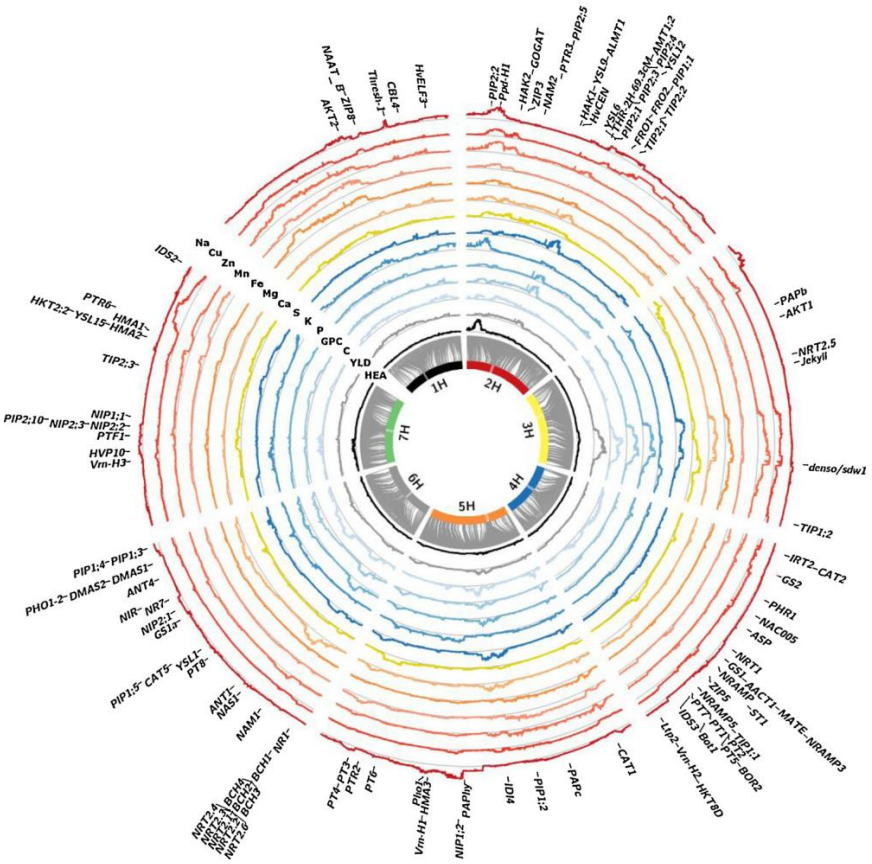

Fig. 4. Results of the single marker regression analysis across the studied traits in Dundee. Barley chromosomes are indicated as colored bars on the inner circle, and centromeres are highlighted as transparent boxes. Grey connector lines represent the genetic position of SNPs on the chromosomes. Each track represents one trait, and these are (from inside to outside) HEA, YLD, C, GPC, P, K, S, Ca, Mg, Fe, Mn, Zn, Cu and Na. Trait abbreviations are given in Supplementary Table 3. The colored tracks display the negative common logarithm of the p-values, and the grey line shows the baseline. For exact p-values, see Supplementary Table 10. Peaks of a track indicate effects associated with the respective chromosome region. Candidate genes are depicted outside the circle. The figure is based on BLUEs across years and treatments.

partly match those observed in our survey, since we could also detect increases in GPC, Fe and Zn, as well as decreases in yield in HEB-YIELD lines carrying the wild allele, although not significant for Fe and Zn. Therefore, lines with the wild allele seem to carry a functional version of *NAM-1*, because the functional protein is associated with higher protein concentrations [123].

3.6.4. Additional genomic regions

Also the short arm and pericentromeric region of 5H exerted significant effects on nutrient concentrations. The first-mentioned region might be promising, since lines carrying the wild allele were characterized by higher concentrations of C, GPC, P, Mg, Fe, Mn, and Zn, without a distinct reduction in yield (Tables S10 & S11). Such loci might be valuable, since they could work as correlation breakers between yield and quality. It is known that *CAT1* is located in this area. CAT1 belongs to a family of cationic amino acid transporters (CAT) that were first identified in Arabidopsis [127,128]. CATs mainly function as amino acid transporters and are expressed in various plant tissues [127], which may be an indication for the detected effect on GPC.

The pericentromeric region of 5H showed pronounced effects on S (Tables S10 & S11), which might point to an aspartate/tyrosine/aromatic aminotransferase (*IDI4*) as possible candidate gene. *IDI4* is located in the centromere region of 5H and catalyzes the final step of the synthesis of the sulfurous amino acid methionine [129].

Another interesting finding was the impact on a multitude of traits originating from the pericentromeric region of 2H. In both locations the traits YLD, GPC, P, S, Ca, Mg, and Zn were significantly affected, whereupon the wild allele increased all of them except YLD. So far we could not identify a reliable candidate gene causing the effects, and it would be worth to have a closer look at this region in follow-up studies.

4. Conclusions

In summary, our results clearly support the existence of a negative relationship between quantity and quality in the barley HEB-YIELD population, expressed as a loss of nutritional value of grains with increasing yields. This relationship is well-known from modern crops and leads to an eminent dilemma [16–18] because breeding for human food and animal feed demands to simultaneously increase both grain quantity and quality [2,3,9,10]. One approach to improve both complexes may be to continue to target grain yield as main breeding goal, which would indirectly also increase nutrient yields, since we could show that grain yield is highly positively correlated with them. However, this would further dilute the nutrient concentrations and reduce the nutritional value of cereal grains [18]. Therefore, yield improvements, which are necessary to supply the growing world population, ought to be reached without loss of quality through the identification of correlation breakers [109]. Here, we showed that HEB-YIELD offers a large amount of genetic variation for a multitude of nutrients, which can be directly used in crossings and for the identification of genes controlling nutrient concentration in the grain. Wild barley might harbor alleles that increase the nutritional value without yield reductions and function as correlation breakers, as indicated by interesting genomic regions like the one on the short arm of chromosome 5H. Consequently, we recommend to dig deeper into the genetic regulation and identification of exotic alleles controlling nutrient concentration traits in follow-up studies with wild barley. Ultimately, promising wild barley alleles could be introgressed into elite material. In addition, the expression of effective wild barley alleles could be locally regulated, for example, by genetic engineering, as recently applied in wheat and barley [105,106].

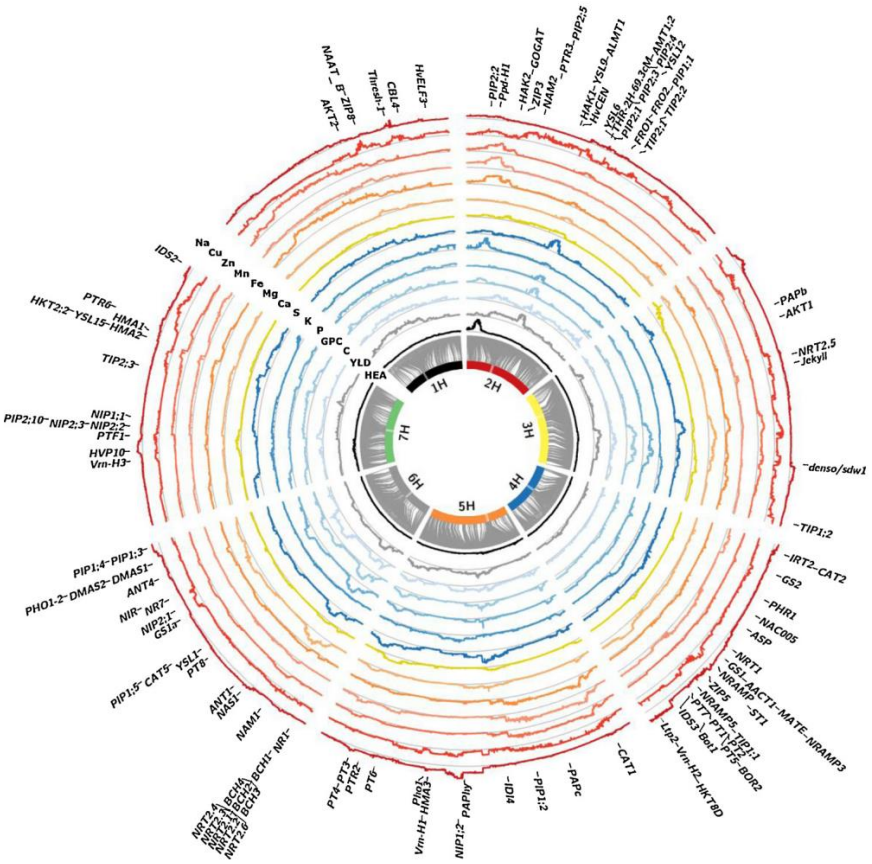

Fig. 5. Results of the single marker regression analysis across the studied traits in Halle. Barley chromosomes are indicated as colored bars on the inner circle, and centromeres are highlighted as transparent boxes. Grey connector lines represent the genetic position of SNPs on the chromosomes. Each track represents one trait, and these are (from inside to outside) HEA, YLD, C, GPC, P, K, S, Ca, Mg, Fe, Mn, Zn, Cu and Na. Trait abbreviations are given in Supplementary Table 3. The coloured tracks display the negative common logarithm of the p-values, and the grey line shows the baseline. For exact p-values, see Supplementary Table 10. Peaks of a track indicate effects associated with the respective chromosome region. Candidate genes are depicted outside the circle. The figure is based on BLUEs across years and treatments.

Declaration of interest

The authors declare that they have no competing interests.

Author contributions

MW conducted the field trials in 2015 and 2016 in Halle, gathered and analyzed the phenotypic data of the two locations, created the figures, and drafted the manuscript. WT, HB and AF planned and conducted the field trials in 2015 and 2016 in Dundee. AZ organized the wet chemistry analysis and drafted the manuscript. EP supported the candidate gene analysis and drafted the manuscript. KP planned the project, acquired funding, coordinated the collaboration between the project partners and drafted the manuscript. AM planned and coordinated the field trials in 2015 and 2016 in Halle, supported the analysis of the phenotypic and genotypic data and drafted the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2018.12.030.

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II.III. Wet chemistry vs. hyperspectral imaging

„Defining parameters for model optimization in grain nutrient predictions in barley via hyperspectral imaging"

By

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RESEARCH ARTICLE

Optimizing the procedure of grain nutrient predictions in barley via hyperspectral imaging

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Abstract

Hyperspectral imaging enables researchers and plant breeders to analyze various traits of interest like nutritional value in high throughput. In order to achieve this, the optimal design of a reliable calibration model, linking the measured spectra with the investigated traits, is necessary. In the present study we investigated the impact of different regression models, calibration set sizes and calibration set compositions on prediction performance. For this purpose, we analyzed concentrations of six globally relevant grain nutrients of the wild barley population HEB-YIELD as case study. The data comprised 1,593 plots, grown in 2015 and 2016 at the locations Dundee and Halle, which have been entirely analyzed through traditional laboratory methods and hyperspectral imaging. The results indicated that a linear regression model based on partial least squares outperformed neural networks in this particular data modelling task. There existed a positive relationship between the number of samples in a calibration model and prediction performance, with a local optimum at a calibration set size of ~40% of the total data. The inclusion of samples from several years and locations could clearly improve the predictions of the investigated nutrient traits at small calibration set sizes. It should be stated that the expansion of calibration models with additional samples is only useful as long as they are able to increase trait variability. Models obtained in a certain environment were only to a limited extent transferable to other environments. They should therefore be successively upgraded with new calibration data to enable a reliable prediction of the desired traits. The presented results will assist the design and conceptualization of future hyperspectral imaging projects in order to achieve reliable predictions. It will in general help to establish practical applications of hyperspectral imaging systems, for instance in plant breeding concepts.

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Introduction

Cereals form the basis of human nutrition all over the world, since they provide us with our daily food [1,2]. Their grains do not only contain energy in form of carbohydrates, but also proteins, fiber and nutrients [3–6]. They represent a source for processed food products like wheat flour for baking [7] and barley malt used in the beverage industry [6,8]. Moreover, cereals supply livestock breeding with fodder, which has specific quality requirements for animal nutrition [9,10].

Barley (*Hordeum vulgare* ssp. *vulgare*) is one of these cereals and the world's fourth most important cereal crop regarding production [8,11]. It serves mainly as source for fodder, malt and food [6,8]. In each of these uses, barley and processed barley products need to meet prescribed quality requirements [12–14]. In this regard the protein concentration of mature grains defines if barley can be used for malt (10–12% grain raw protein concentration) or fodder (no restrictions) production [12,15]. Another example would be the mineral content or rather nutritional value of barley grains, which is important if humans or animals consume barley. For example, about one billion people suffer from low intakes of proteins and nutrients, especially iron, zinc and calcium [16–18].

The majority of grain quality measurements is based on wet chemistry analysis, like the determination of the nutritional value of seeds or the digestibility of animal fodder. The results obtained from these techniques are precise and trustworthy, however the methods themselves are time-consuming, labor-intensive and expensive [19–21]. In addition, in most cases they are destructive, i.e. the plant material (e.g. seeds) is destroyed during the analysis. These drawbacks prevent the standardized application of quality analysis of high numbers of genotypes in breeding programs, especially in early stages of selection [22,23]. Spectroscopy-based technologies have been successfully implemented in the last decades to circumvent the stated drawbacks, and are frequently applied by plant breeders and scientists [19,24,25]. The most common technique is near infrared spectroscopy (NIRS), which is based on the emission of near infrared radiation (750–2500 nm) that is absorbed by O-H, C-H, C-O and N-H bonds, the main compounds of plant tissues [19,26], resulting in a unique reflection spectrum for each compound. Therefore, the specific chemical composition of the analyzed material results in a spectral fingerprint [19,26].

A major constraint of NIRS is the missing information about the exact location of individual chemical components inside the sample. This can be resolved by combining spectroscopic and vision techniques, officially termed as hyperspectral imaging (HSI) [27,28]. A hyperspectral image consists of a two-dimensional (classic) image and spectral data as a third dimension. Both are obtained by hyperspectral camera systems creating a so-called three-dimensional data cube [29], which contains the information about the locally different spectral reflectance [27,28]. It should be noted that both NIRS and HSI are much more complex and can only briefly be introduced here (for details about NIRS see Foley et al. [19] and Cen and He [26]; for HSI see ElMasry and Sun [27] and Park and Lu [28]). Both technologies have already been used in a multitude of different fields [30,31], including grain quality analysis [32–34].

However, the spectral data acquisition of NIRS and HSI cannot stand alone, since both need the calibration of models to relate the measured spectra with phenotypic values (e.g. ingredient concentrations or digestibility) [26,27,35,36]. The calibration models are based on a smaller number of samples, which often is a sub-sample of the whole investigated dataset. These samples should ideally reflect the range of variation of the investigated dataset and are analyzed using standard laboratory methods [37]. To a high extent, the quality of the calibration defines the accuracy and precision of predicting the values of the trait of interests by

spectral technologies [19,26,27,35,36]. One open question is how to size the calibration dataset to obtain high prediction accuracy while keeping wet chemistry costs low.

The specific objective of the present study was the examination of different calibration model designs and their impact on prediction performance of hyperspectral imaging as high-throughput tool for grain quality analysis using the wild barley population HEB-YIELD [38]. Therefore, we investigated the protein and nutrient concentrations of mature grains via wet chemistry analysis (ICP-OES) and hyperspectral imaging at two European locations in two successive years. The hyperspectral imaging results have been compared to those originating from wet chemistry analysis. Several regression models, calibration set sizes and calibration set compositions have been tested to evaluate the impact of calibration quality on phenotypic value estimation.

Materials and methods

Plant material

HEB-YIELD [38], a subset of the wild barley nested association mapping (NAM) population Halle Exotic Barley-25 (HEB-25, [39]), was used in this study. HEB-25 originated from crossing 25 diverse wild barley accessions (*Hordeum vulgare* ssp. *spontaneum* and *H. v.* ssp. *agriocrithon*) with the German elite spring barley cultivar Barke (*Hordeum vulgare* ssp. *vulgare*, released in 1996 by breeder Breun). HEB-25 comprises 1,420 BC_1S_3 derived lines (backcrossed with Barke), grouped into 25 families (for more details see Maurer et al. [39]).

The HEB-YIELD subset consists of 48 HEB-25 lines that were selected from HEB-25 to ensure good threshability and the absence of brittle rachis, whereby enabling accurate yield estimation in field trials.

Field trials

The HEB-YIELD population was grown at two locations during two years (2015 and 2016), resulting in four environments. The locations were Dundee (United Kingdom; 56˚28'53.71"N 3˚6'35.17"W) and Halle (Germany; 51˚29'46.05"N 11˚59'29.58"E). At both locations the plants were cultivated under regular fertilization and under nitrogen deficiency together with local checks in four replications. Under nitrogen deficiency the lines received no additional mineral N fertilizer. The difference between both treatments regarding N were among 60 and 70 kg/N per hectare in both years by considering the results of the N_{min} analysis, which was performed in early spring prior to sowing to determine the availability of N for the HEB-YIELD lines. A detailed description is given in Wiegmann et al. [40].

The studies were conducted on land owned by the authors' institutions. The research conducted complied with all institutional and national guidelines.

Phenotypic data

In this study grain elemental concentrations of six agronomically important traits were investigated, including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), and zinc (Zn). A list of these traits is given in S1 Table, including their method of measurement and in which location and year the traits were scored.

In a previous study, based on the same wet chemistry data, it could be shown that the nutrient concentration of grains was not influenced by the conducted N treatment [40]. Therefore, the results of the present paper are based on merged data from both N treatments.

Standard descriptive statistics on raw phenotype data of the investigated traits (see above) were calculated and the coefficient of determination (CV) was defined as $\frac{standard\ deviation}{arithmetic\ mean}$.

Hyperspectral image recording

Hyperspectral images have been taken in a unique high-throughput phenotyping platform, whose main components are: (1) object plate, (2) white reference, (3) light source, (4) HSI camera and (5) electronically controlled railed carriage (S1 Fig). The phenotypic platform was developed in collaboration with the Fraunhofer Institute for Factory Operation and Automation (IFF).

For achieving a low and homogenous reflection background across the investigated wavelengths the object plate was coated in black fleece. As white reference the Zenith Lite diffuse reflectance target (SphereOptics GmbH, Herrsching, Germany) with a reflection of 95% (spectralon) was used and scanned for each grain sample. The grain samples haven been illuminated through two 150 W quartz halogen lamps in combination with two reflectors to avoid a loss of radiation intensity. These lamps were positioned in a 45˚ and 135˚ angle relative to the horizontally placed grains on the object plate. In addition, the image acquisition was conducted in a shaded room without external light sources, except the mentioned halogen lamps and the phenotyping platform was covered with black molleton. The heart of the whole platform was the HySpex SWIR 384 hyperspectral pushbroom camera (HySpex, Skedsmokorset, Norway), which had the capacity to encompass a spectral range of 970 to 2500 nm (near-infrared region) with 288 bands. These bands were equally spaced across the spectral range. The camera was equipped with a lens of 30 cm fixed focal length. Both the HSI camera and the light source were mounted on an electronically moveable railed system with a distance of 30 cm to the grain sample underneath of it. With this setup 16 Bit digitized high resolution reflectance data with 384 spatial pixels in line at a maximal achievable frame rate of 400 Hz were obtained.

The spectral data for the 1,593 grain samples investigated in this study have been obtained through the above described phenotyping platform and all samples were subsequently analyzed via wet chemistry as described in the next chapter.

Nutrient analysis via wet chemistry

After air drying the harvested grains for two weeks, 6-8 g of grains of each plot were ground and homogenized using the mixer mill MM 400 (Retsch GmbH; Haan, Germany).

The dry matter concentration (DM) of each sample was determined after drying the barley flour for 3 hours in a drying cabinet at 105˚C (method 3.1 modified [40]).

The element N was measured with a CNS analyzer (vario EL cube; Elementar Analysensysteme, Langenselbold, Germany), which is based on combustion analysis [40].

For determination of the macronutrients (P, K & Mg) and micronutrients (Fe & Zn) inductively coupled plasma—optical emission spectrometry (ICP-OES) was used (Varian 715-ES ICP-OES; Varian, Palo Alto, California, USA). For more details about wet chemistry analysis, see Wiegmann et al. [40].

Nutrient analysis via hyperspectral imaging

Hyperspectral image cubes were processed by the automated workflow system HawkSpex Flow developed by the Fraunhofer IFF written in Matlab (Mathworks Inc.). In order to obtain reflectance values, the white target was automatically marked and extracted. Reflectance calculation was performed using

$$R_\lambda = \frac{I_\lambda - I_\lambda^{DC}}{I_\lambda^{W} - I_\lambda^{DC}}$$

where I_λ is the image pixel intensity at wavelength λ, I_λ^{DC} the intensity when measured with

closed shutter ("dark current") and I_λ^W being the intensity while recording the spectralon device. For a number of images a Neural Gas algorithm [41] was used to cluster the principal material groups in the image (spectralon, table surface, grains). The cluster mask representing the grain material was manually selected and corrected. These segmentation masks defined the identity of foreground (grain) and background (spectralon, table surface) pixels. A Radial Base Function (RBF) Neural Network [42] was then trained as classifier to separate foreground and background. This classifier was then applied to all grain images and yielded a robust and fully automated separation of grains and background.

Pixels representing grain material were then collected and their respective spectrum per grain image was averaged. These average spectra were used as input for a regression model, where a nutrient served as target value. In order to test the effect of different sample sizes, several validation schemes were performed with 5%, 10%, 20%, 40%, 60% or 80% of the target values being randomly included in the calibration set. Sample selection was independent of genotype replications, but stratified for the treatment (1:1). In each validation round, the given percentage of samples was then used to calibrate the regression model while the remaining samples served as test samples. In total, 100 validation rounds with the respective random split were calculated. Additionally, a leave-one-out scheme was used where in each validation round one sample is left out of the training set (= N-1; for simplicity referred to as 99%). In this scheme, the number of samples in a particular set determines the number of validation rounds in the modelling. In the leave-on-out scheme, no random sample drawing is performed.

As performance measure for prediction, the coefficient of determination (R^2) was used. R^2 was defined as the squared Pearson correlation coefficient:

$$R^2 = \left(\frac{\sum_{i=1}^{n} (y_i - \bar{y})(t_i - \bar{t})}{\hat{\sigma}_y \hat{\sigma}_t} \right)^2$$

where y_i is the nutrient prediction for sample i, while t_i is the target (true) nutrient value with $\bar{y}$ and $\bar{t}$ being their respective averages as well as $\hat{\sigma}_y$ and $\hat{\sigma}_t$ being their respective standard deviations. A perfect prediction is achieved with an R^2 of 1.0. The threshold of R^2 values, above which a sufficient prediction is achieved, is debatable.

As regression models, a Partial Least Squares (PLS) Regression Model, which is a basic method in optical chemometrics [43], along with two neural network types, a Radial Base Function with Transfer Learning (tRBF) Neural Network [44] and a Multi-Layer Perceptron Network [45] were applied (for more details see Table 1).

A PLS model finds a linear regression model by projecting the predicted variables and the observable variables to a new space similar to a principal component analysis (PCA). In contrast to a PCA, PLS is finding hyperplanes of maximum variance between the response or target value and independent or observed variables. PLS model parameters are found by least squares method. The number of PLS components was manually set to 20.

Table 1. Regression model details.

Model	Hyperparameters	Learning Rule
Partial Least Squares (PLS)	PLS Components = 20	Method of the smallest squares
Radial Base Function Network with Transfer Learning (tRBF)	Radial Basis Function = 20 Metric = Euclidean Distance	Scaled Non-Linear Conjugate Gradient; Matlab Package minFunc
Multi-layer Perceptron (MLP)	Two Hidden Layers Hidden Layer 1 = 30 Neurons Hidden Layer 2 = 10 Neurons Hidden Layer Activation = tansig Output Layer Activation = linear	Levenberg–Marquardt; Matlab Neural Network Toolbox

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Data-driven learning methods like Artificial Neural Networks (tRBF and MLP) try to model a system behavior not by formulating a physical model but parameterizing a general purpose numerical structure. In general, an Artificial Neural Network derives its idea from the information and learning process in the human brain, where a large number of simple processing units are linked together by weighted connections. Technically, a neural network is a universal function approximation system. A numerical model generates an output from an input via structure neurons. The output is compared to a target value (or ground truth value) and an error value is calculated, the so-called loss function. The learning parameters then adjust the weighted connections of the network iteratively so that the error produced by all training samples is minimal. In that way, a generic numeric function is fitted to an input/output problem and generates in our case a regression model for predicting nutrient concentration (output) from spectral reflectance measurements (input) without the need to model a physical process how a reflectance is produced by a nutrient concentration. The parameters of the applied tRBF and MLP neural networks are found by numerically optimizing the objective function of mean squared error (MSE) between target and prediction value. Optimization is performed using a gradient descend approach and stopped if a number of epoch (1000) is reached or the MSE converges, e.g. changes in MSE fall below a defined threshold of 1e-05.

The tRBF models the dataspace as a weighted mixture of Gaussian kernel functions calculated via distance calculation of the input sample towards prototypical patterns retained in the model, while MLP tries to model the data via the use of hyperplanes.

Calibrating a number of different regression models is a typical approach in machine learning since it is difficult to assess the nature of a high-dimensional dataspace and to decide whether the systematic relationship between the spectrum and the nutrient is linear (PLS) or non-linear (tRBF, MLP).

Modelling was performed on separate datasets for single environments, as well as for a two-year model per location and across all four environments. In order to test the transferability of the models, samples that were not used for model training were predicted and the prediction quality was assessed with the R^2 measurement as described above.

Cost benefit analysis

In order to estimate the relative prediction performance gain with increasing sample number, a cost benefit analysis was carried out between two consecutive calibration set sizes, each based on the following formula,

$$\frac{\Delta prediction\ performance}{\Delta sample\ number}$$

with Δ indicating the difference between two consecutive calibration set sizes with regard to prediction performance (e.g. $R^2_{10\%}$—$R^2_{5\%}$) and sample number (e.g. $N_{10\%}$—$N_{5\%}$), respectively.

Statistical analyses

SAS 9.4 (SAS Institute Inc., Cary, NC, USA; [46]) was used to estimate variance components for each environment separately with *PROC VARCOMP* by including the random factor genotype to explain a trait. Based on the estimated variance components repeatabilities (rep) were calculated within each environment:

$$rep = \frac{V_g}{V_g + \frac{V_\varepsilon}{R}}$$

, where

V_g = genotype variance (based on 48 genotypes)
V_r = residual variance
R = number of replicates (4)

The different regression models and calibration set compositions have been investigated for statistical significance regarding their prediction performance through the results of a one-factorial (factors regression model and calibration set composition, respectively) ANOVA (R package "stats" 3.6.1) and a subsequent Tukey's test ([47]; R package "agricolae" 1.3.1). A Fisher's z transformation ([48]; R package "psych" 1.8.12) was applied over Pearson's correlation coefficients of prediction performance to account for non-normal distribution. We checked for homogeneity of phenotypic variances between the random sampling of the three regression models (PLS, MLP, tRBF) to rule out that differences in prediction performance between them were caused by differences in phenotypic variances by applying Fligner-Killeen tests ([49]; R package "stats" 3.6.1).

All figures were created using R 3.6.1 [50] with the package "ggplot2" 3.2.0 [51], except S14 Fig, which was created with SAS *PROC SGPANEL*.

Results and discussion

Phenotypic data

Every spectral-based technology depends on measuring a subset of the samples via wet chemistry analysis to generate a calibration model to link the spectra with the phenotypic values determined in the laboratory [27,35,36,52]. In the present study the full set of all 1,593 samples from the wild barley introgression population HEB-YIELD, grown in Dundee (United Kingdom) and Halle (Germany) in 2015 and 2016, has been measured using wet chemistry to determine six grain nutrients, including four macronutrients (N, P, K & Mg) and two micronutrients (Fe & Zn) (S2 Table). The majority of these traits showed a considerable amount of variation indicated by the coefficient of variation (CV), which ranged from around 6% for Mg in Halle 2015 to more than 23% for Fe in Dundee 2016 (S2 Table). Moreover, the average repeatability of 0.93 for the six nutrient traits indicates that the effect of the genotype on these traits is high and the residual variance is comparatively low, also hinting on trustworthy wet chemistry measurements (S2 Table).

Prior to the wet chemistry analysis, the hyperspectral reflectance of each grain sample has been captured via HSI by using the same grains that were utilized for subsequent wet chemistry analysis. Finally, all 1,593 samples were analyzed via wet chemistry (S3 Table) and hyperspectral imaging to determine grain nutrients.

The resulting dataset was used in a case study to investigate the impact of different calibration models on prediction performance of hyperspectral imaging for nutrients in mature barley grains. The calibration models varied based on the applied regression model, the number of samples used for the calibration set, as well as the sample selection for the calibration sets, which was either conducted within a single environment, across years, or across environments. The coefficient of determination (R^2) serves as measure for the prediction performance of the calibration models throughout the study.

Comparison of regression models

Independent of the material (e.g. grains, food or landscapes) that is scanned by a HSI camera system, the resulting spectra need to be linked to a target trait (e.g. phosphorus content, free fatty acids or soil type) by applying an adequate regression model [27,35,36]. Three regression models, based on multi-layer perceptron (MLP), radial base function network with transfer

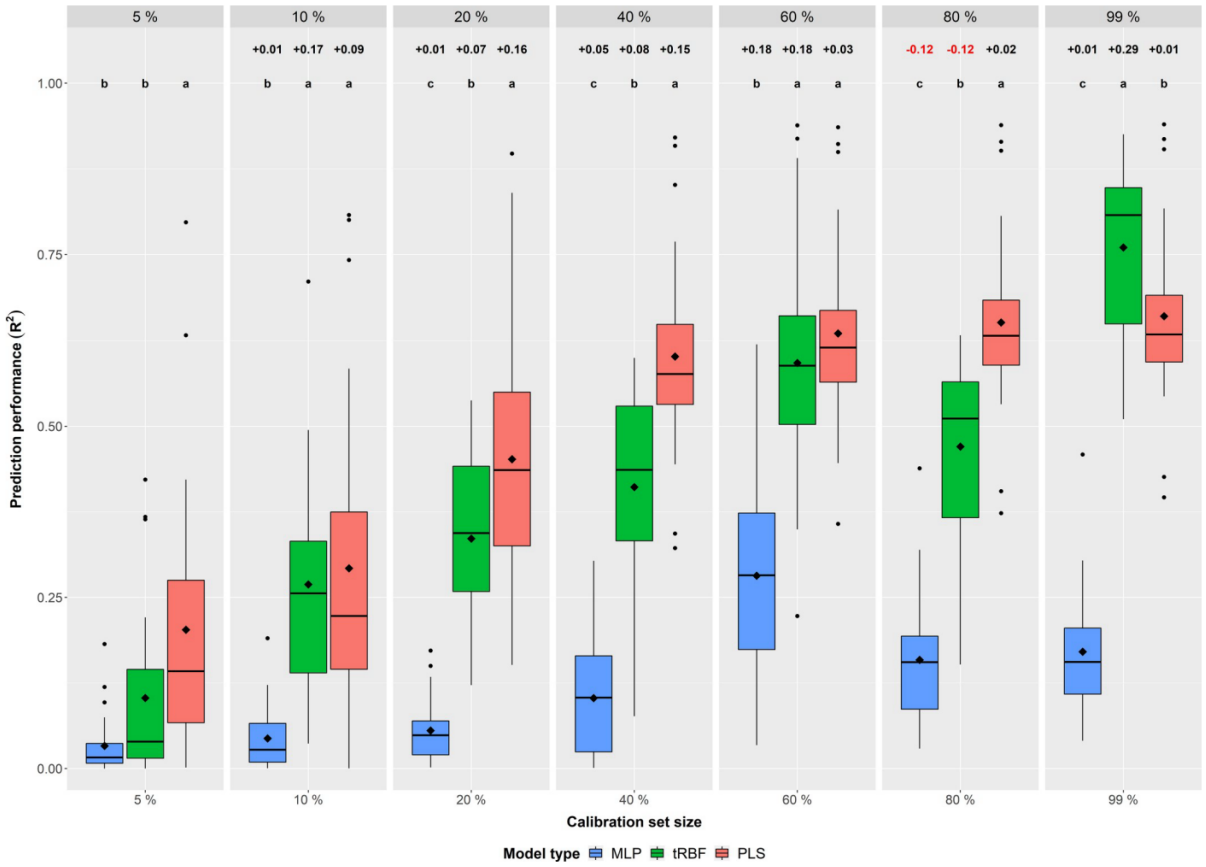

Fig 1. Regression model comparison—Across environments—Across traits. Comparison of the investigated regression models in regard to prediction performance (R^2) across the four environments (DUN15, DUN16, HAL15 & HAL16) and the six nutrient traits (N, P, K, Mg, Fe & Zn) for different calibration set sizes from 5% to 99%. The color of the boxplots differentiates the three different model types MLP (multi-layer perceptron, blue), tRBF (radial base function network with transfer learning, green) and PLS (partial least squares, red). The diamonds inside the boxes indicate the arithmetic mean. Letters (a, b, c) in the upper part of the figure indicate significant ($P<0.05$) differences between the models based on a Tukey test (S4 Table). Furthermore, numbers above the letters indicate the change in prediction performance compared to the next smaller one.

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learning (tRBF) and partial least squares (PLS), were tested to evaluate if the model type affects prediction performance of grain nutrients.

In accordance to a multitude of spectral-based studies originating from various fields of research [53–57], the choice of a suitable calibration model is also critical for predicting grain nutrients.

The combined data of the four environments, averaged across all six nutrients, revealed a clear ranking of the regression models, where the best predictions were achieved with PLS followed by tRBF and MLP (Fig 1). This trend was also valid by looking at the results for single environments (S2 Fig; S3 Fig; S4 Fig; S5 Fig) and single nutrients (S6 Fig). A Tukey test confirmed the low performance of the MLP model, since its predictions were significantly below the average prediction performances of the two remaining models (S4 Table). The predictions made with the tRBF model were in all calibration set sizes, except the largest one (99%), below the average of PLS, although statistically not always significant (S4 Table).

Furthermore, the regression models can be differentiated based on their computing demand, which increases in the following order: PLS < tRBF < MLP (on average 0.2 s < 20 s < 50 s per single model in our dataset). It should be noted that the computing demand to generate the calibration models is substantial, even if high computing performance systems are available. Therefore, it represents an additional factor in choosing an adequate model.

Due to the good prediction performances of the PLS model and the lowest computing demand all following results are exclusively based on PLS (results of MLP and tRBF are available in Supplementary Tables). The PLS model is the basic model in optical chemometrics [43] and a well-suited tool for the analysis of spectral data [58,59]. It has been successfully applied in various fields of spectroscopy [60–62]. However, one should note that the suitability of certain regression models is highly dependent on the dataset for the task at hand and an approach of testing different regression methodologies should be followed. In this context it should also be noted that if larger wet lab datasets were available machine learning methods like MLP and tRBF will most likely benefit, giving the possibility of reaching higher predictive abilities.

Comparison of calibration set sizes

In the present study all samples were entirely analyzed via wet chemistry, which enabled to flexibly adjust calibration set sizes to find the minimal size for achieving good predictions. As already indicated in Fig 1, the size of a calibration set affects the quality of the calibration model and, finally, the prediction performance of HSI. If money and time would not be limiting factors the best way to obtain trustworthy grain ingredient data would certainly be the analysis of all samples by standard laboratory methods [19–21]. In reality, however, an ideal calibration set has to be defined based on a cost-benefit analysis. On the one side a calibration set needs to be large enough to enable reliable predictions, on the other hand it should not be larger than necessary to avoid excessive wet chemistry costs. Esteve Agelet and Hurburgh [52] indicated that the choice of the right calibration set is frequently underestimated, even though it defines the quality of spectroscopy-based analyses. Therefore, we created individual calibration models with seven different sample sizes (5% 10%, 20%, 40%, 60%, 80% and 99%, reflecting an approximate sample number of n≈20, 40, 80, 160, 240, 320 and 400 in each environment, respectively) for the six nutrient traits. On average, in each environment an enhancement of the calibration set resulted in an improvement of the prediction performance. This increase can be described through a regression based on the natural logarithm in all four environments (mean R^2 of 0.96; Fig 2; S5 Table).

The effect of the calibration set size has also been investigated for each nutrient across the four environments (Fig 3; S5 Table), as well as within each of them separately (S5 Table; S7 Fig; S8 Fig; S9 Fig; S10 Fig). For all nutrients the same trends regarding the calibration set size effect on prediction performance could be observed. By far the best values could be obtained for N, reflecting the grain raw protein content, which reached R^2 values >0.9. For this nutrient, a calibration set of 40 samples (10%) was sufficient to achieve reliable measurements with an average R^2 of 0.65. The good predictions for N are in agreement with trustworthy prediction of N by using NIRS [35,63,64]. For instance, Velacso and Möllers [63] found an R^2 of 0.94 between NIRS and combustion analysis for protein content in rapeseed. The nutrients P, K, Mg, Fe and Zn were characterized by intermediate prediction performances, indicated by mean R^2 values of >0.48 at a calibration set size of n = 160 (40%).

The effect of the calibration set size on prediction performance was different for each trait. However, a general pattern existed that appreciable improvements were possible until a calibration set size of 160 samples (40%) was reached (Fig 3; S11 Fig; S12 Fig; S13 Fig). From this stage on a plateau was reached and each further added sample could only marginally increase

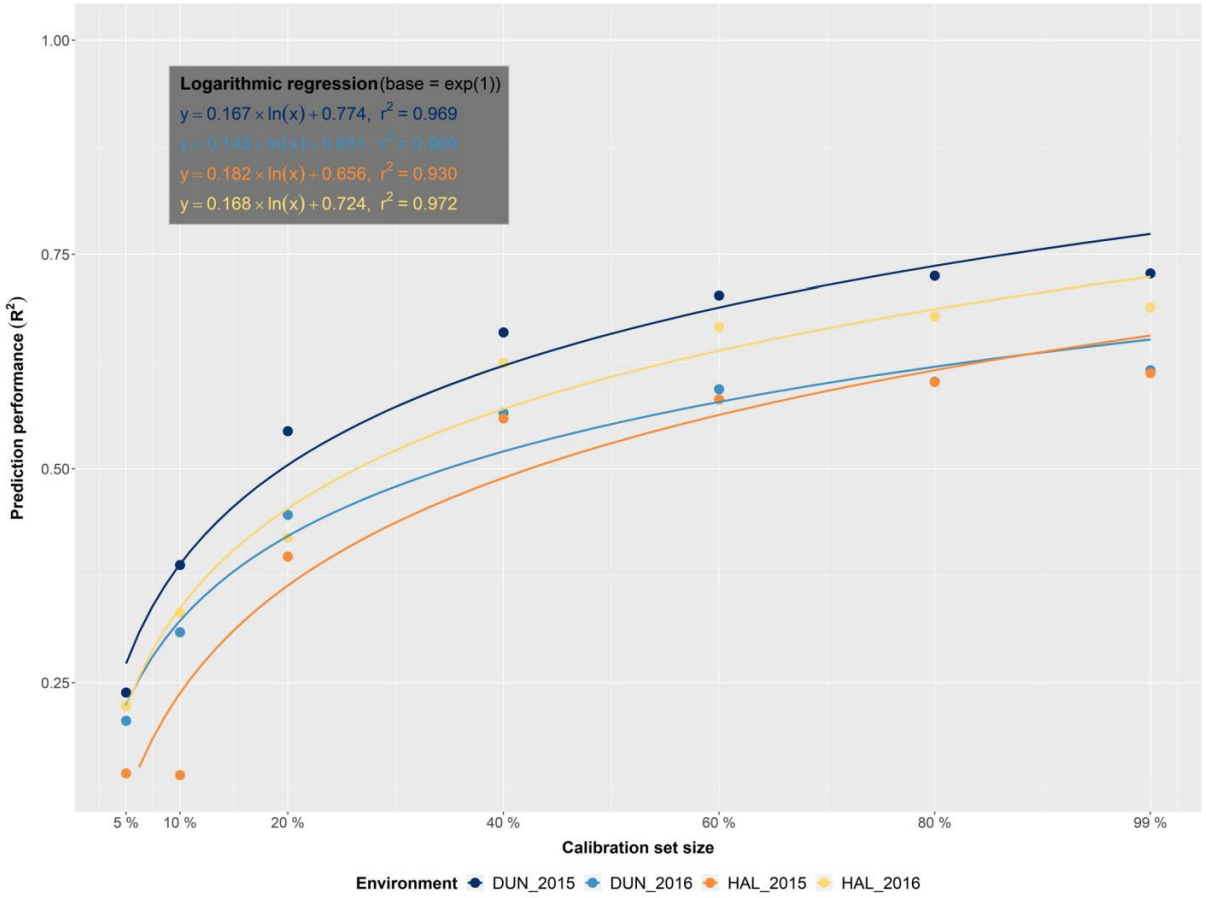

Fig 2. Calibration set size comparison—Within environments—Across traits. Impact of calibration set size on prediction performance (R^2) in each of the four environments (DUN15 = dark blue, DUN16 = light blue, HAL15 = orange, HAL16 = yellow) across the six nutrient traits (N, P, K, Mg, Fe & Zn). A logarithmic function was fitted, which indicates the gain in prediction performance (R^2) with increasing calibration set sizes. The formulas of these four functions are shown in the upper left corner.

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R^2 by ≈0.0004 (S6 Table). This finding may be explained by the fact that the variation of the samples in the calibration set at this stage already adequately reflects the variation of the whole dataset, which is one requirement for valid predictions [37,52]. With increasing calibration set size the range of covered trait values also increases, which might lead to a better predictive model. The high mean correlation coefficient of 0.93 between the trait value range covered by the calibration set and the prediction performance (R^2) confirms this assumption (S14 Fig).

By looking at the impact of calibration set size on prediction performance in each environment individually (S7 Fig; S8 Fig; S9 Fig; S10 Fig; S11 Fig; S12 Fig; S13 Fig), it is frequently observable that the performance fluctuates in smaller calibration sets (5%, 10% and 20%). This is especially pronounced in Halle 2015 for the 10% calibration set size, which gives worse predictions than the 5% calibration set size (S9 Fig). We also observed this in the remaining environments like in Dundee 2015 for Fe (S7 Fig), in Dundee 2016 for K and Fe (S8 Fig) and in Halle 2016 for N, P and Mg (S10 Fig). This observation is unexpected, since in general larger calibration sets should lead to more trustworthy predictions [65]. It may be explained by the

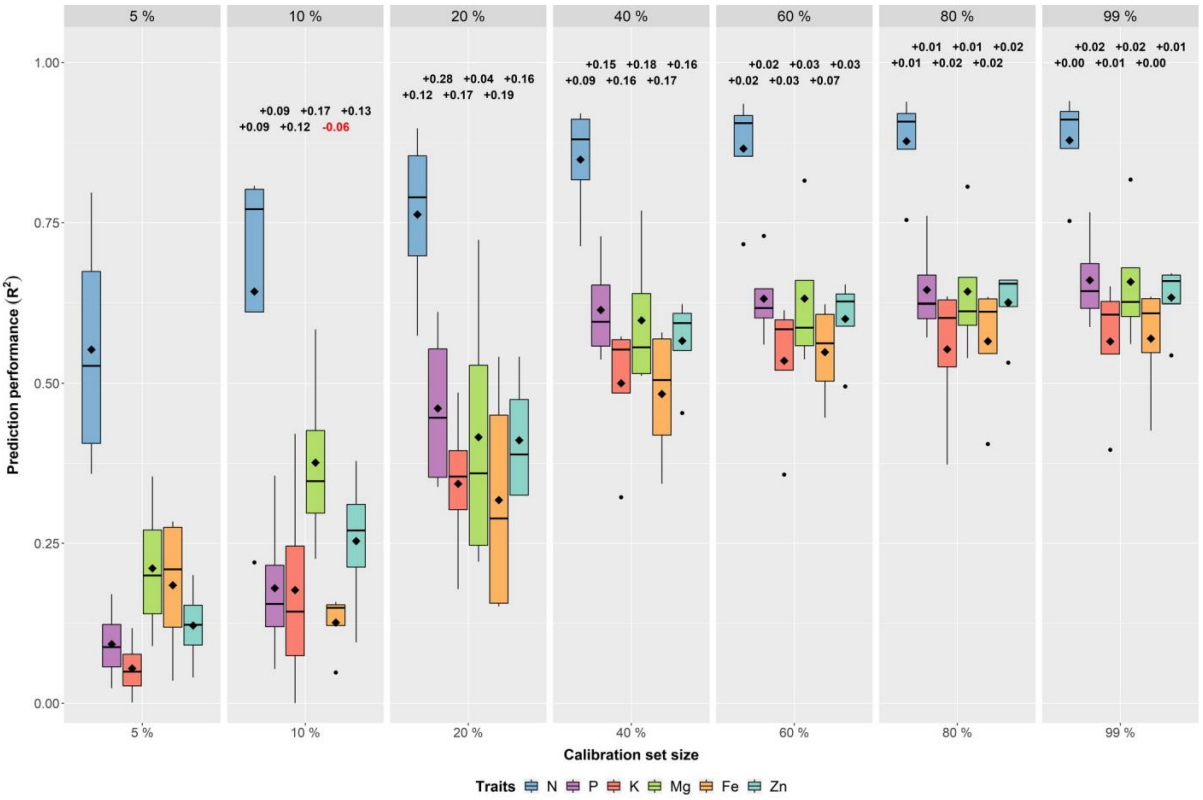

Fig 3. Calibration set size comparison—Across environments—Within traits. Impact of calibration set size on prediction performance (R^2) across the four environments (DUN15, DUN16, HAL15 & HAL16) for each of the six nutrient traits (N, P, K, Mg, Fe & Zn). The color of the boxplots represents the six different traits and the diamonds inside the boxes indicate the arithmetic mean. The numbers in the upper part of the figure indicate the change in prediction performance compared to the next smaller one.

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fact that in small calibration sets the probability is higher that by chance the selected samples do not adequately reflect the variation of the investigated population. The importance of having representative samples in a calibration set is well-known and has already been investigated decades ago [37,66–68]. Also overfitting might play a role in this context, which was observed in small calibration set sizes (0.05 and 0.1), indicating that results gathered from these calibration set sizes should be taken with caution (S5 Table).

However, the general trend that higher calibration set sizes positively influence prediction performance is undisputable and based on the results the recommended calibration set size should be around 160 samples to achieve reliable predictions with an R^2 of 0.5 for P, K, Mg, Fe and Zn, whereas for N already 80 samples are adequate. It should be stated that most measurements related to plant breeding are affected by population-specific effects [69–71], which will also apply to the HSI analysis of grain ingredients. Therefore, the presented results should always be evaluated against the background of the examined wild barley population HEB-YIELD.

Expanding calibration set models

It is well-known that different years and locations impact plant characteristics like height or grain yield [69,72,73], which also holds true for the concentration of nutrients in mature grains in barley [40]. Therefore, calibration models should be recurrently upgraded to increase their

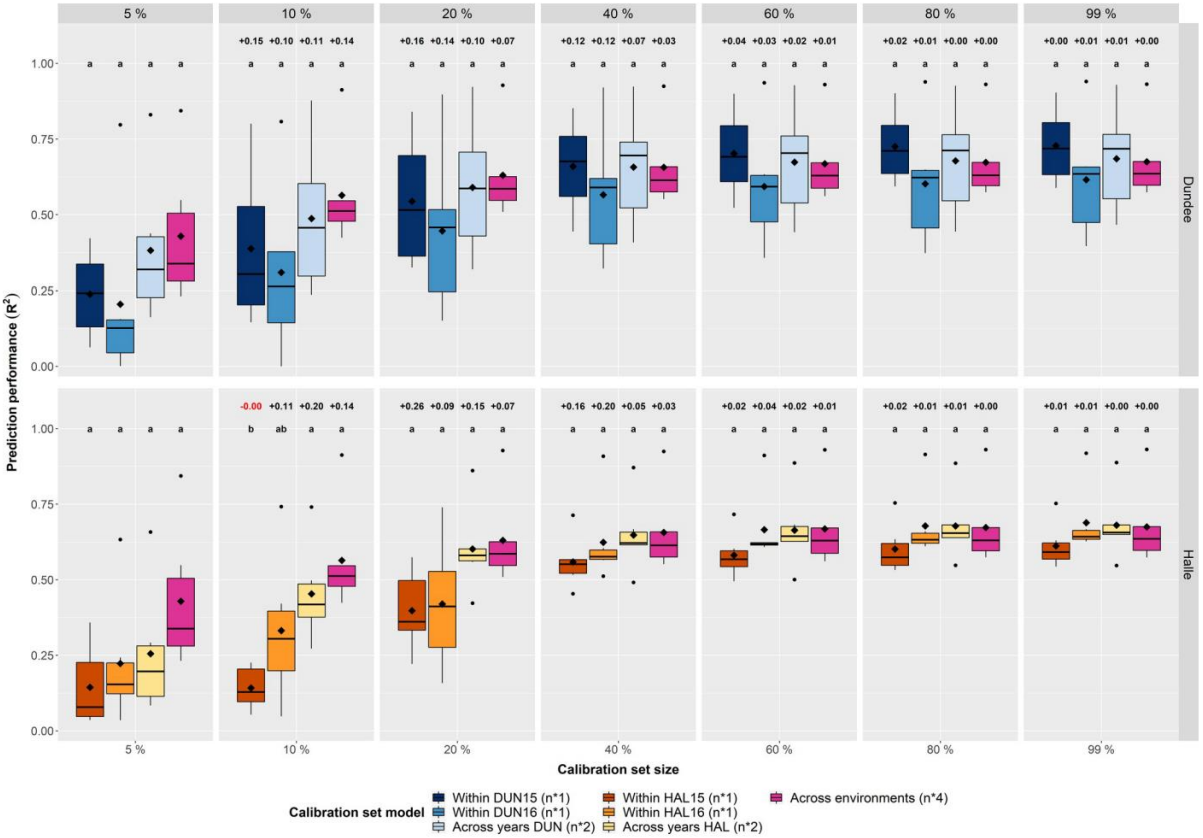

Fig 4. Calibration model comparison—With additional samples—Within environments—Across traits. Comparison of the three calibration set compositions (within environments, across years & across environments) across the six nutrient traits (N, P, K, Mg, Fe & Zn) in Dundee and Halle. The color of the boxplots represents the combination of the different calibration set models and environments. The resulting extension of the total number of samples used for the respective model composition is indicated in parentheses (n*1 = single number of samples, n*2 = duplicated number of samples & n*4 = quadruplicated number of samples). The diamonds inside the boxes indicate the arithmetic mean. Letters (a, b) in the upper part of the figure indicate significant ($P<0.05$) differences between the model compositions based on a Tukey test (S7 Table). Furthermore, numbers above the letters indicate the change in prediction performance compared to the next smaller one.

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flexibility [33,37,68]. The studies of León et al. [74] and Roger et al. [75], conducted in olive fruits and wheat grains, respectively, support the negative impacts of uncontrollable effects (e.g. year) on prediction performance, which can be alleviated by expanding the calibration models through the inclusion of samples from several years.

Therefore, the calibration models have been expanded by duplicating (across years) or even quadruplicating (across environments) the sample number of the calibration sets by using equal sample numbers from each year or each environment. For instance, if in the single environment approach 80 samples were used, 160 were used for the across years and 320 for the across environments approach, respectively. This resembles the common procedure in NIRS where the calibration models are expanded successively by including data from several years and locations [52,76–78]. Both the across years and the across environments approach clearly improved the predictions of grain nutrients, especially in calibration sets with a lower sample size (Fig 4; S5 Table; S7 Table). Furthermore, both approaches clearly reduced the variance of the predictions, as indicated by a lower range as well as smaller coefficients of variation for

sample sizes <160 (S8 Table). By looking at the second smallest calibration set (n = 40) in Halle, the average R^2 was 0.14 in 2015, whereas the mean R^2 was increased to 0.45 and 0.56 when predicting based on the across years approach and the across environments approach, respectively (S8 Table). The extension of the calibration model with data of two years could triplicate the average prediction performance in comparison to the single environment approach Halle 2015, while the across years approach contained 80 samples versus 40 samples in the single environment approach. However, further extension of the model with data from two locations revealed only a smaller increase to 0.56 at a calibration set size of 160. The across environments approach reached its maximum prediction performance in the calibration set containing 40% (n = 640) of the samples with an average R^2 of 0.66. Further sample enhancements hardly impacted prediction, which might be the consequence of little additional variation from the additional samples. Only few nutrients showed better predictions in small calibration set sizes with the single environment models (Fig 5; S5 Table). The results confirm the advantage of adding samples from additional environments to calibration models to improve prediction performance as commonly done in NIRS [52,76–78]. Finally, it should be stated that the generation of such complex calibration models is time-consuming (up to several years) and expensive since a higher number of samples from several environments needs to be analyzed by means of wet chemistry.

Transferability of models

Since model implementation is complex, especially when upgrading it successively, a desirable approach would be to develop only a single robust model, which could be transferred to all kinds of environments without additional efforts (also known as external calibration). The idea of transferring models or keeping them robust over longer times is not new [79] and has been investigated in spectroscopic studies with diverse backgrounds [26,74,80], since it would enable to circumvent the obstacles stated above.

Therefore, we investigated how far our developed models are able to predict each single environment. In a first step each single environment model (e.g. Halle 2015; HAL15) was used to predict the four environments (Dundee 2015, Dundee 2016, Halle 2015 & Halle 2016) to obtain an idea of model transferability. As a result, none of the single environment models could reliably predict another environment except its own (Fig 6; S9 Table). The single environment models never reached R^2 values above 0.5, averaged across the traits, in the non-trained environments. This observation also holds true for each single nutrient, except for N (S9 Table; S15 Fig). It is well-known that N is a reliably predictable nutrient [35,63,64], which is in agreement to the present results where the predictions for N reached R^2 values above 0.5 in the non-trained environments, even in calibration sets with only 10% of the maximum number of samples. However, it should be stated that the predictions considerably varied between calibration set sizes. By expanding the prediction models with samples from a second year (e.g. DUN15 and DUN16 = DUN1516) they were able to predict both years, but still failed to estimate the nutrient concentrations in both years of the other location. The next logical step was to incorporate data from all four environments into one model (DUNHAL1516) and to use this model to predict the nutrient concentrations in the four environments. The outcome was a full model that contains data from all investigated environments that is able to predict the nutrients in a reasonable order in all environments. Interestingly, the four within environment approaches still outperformed the joint model in their own trained environment, though only at higher calibration set sizes.

A transfer of models in the current scope of this study seems difficult. Since only two years and two locations are available, the probability is high that due to variations between

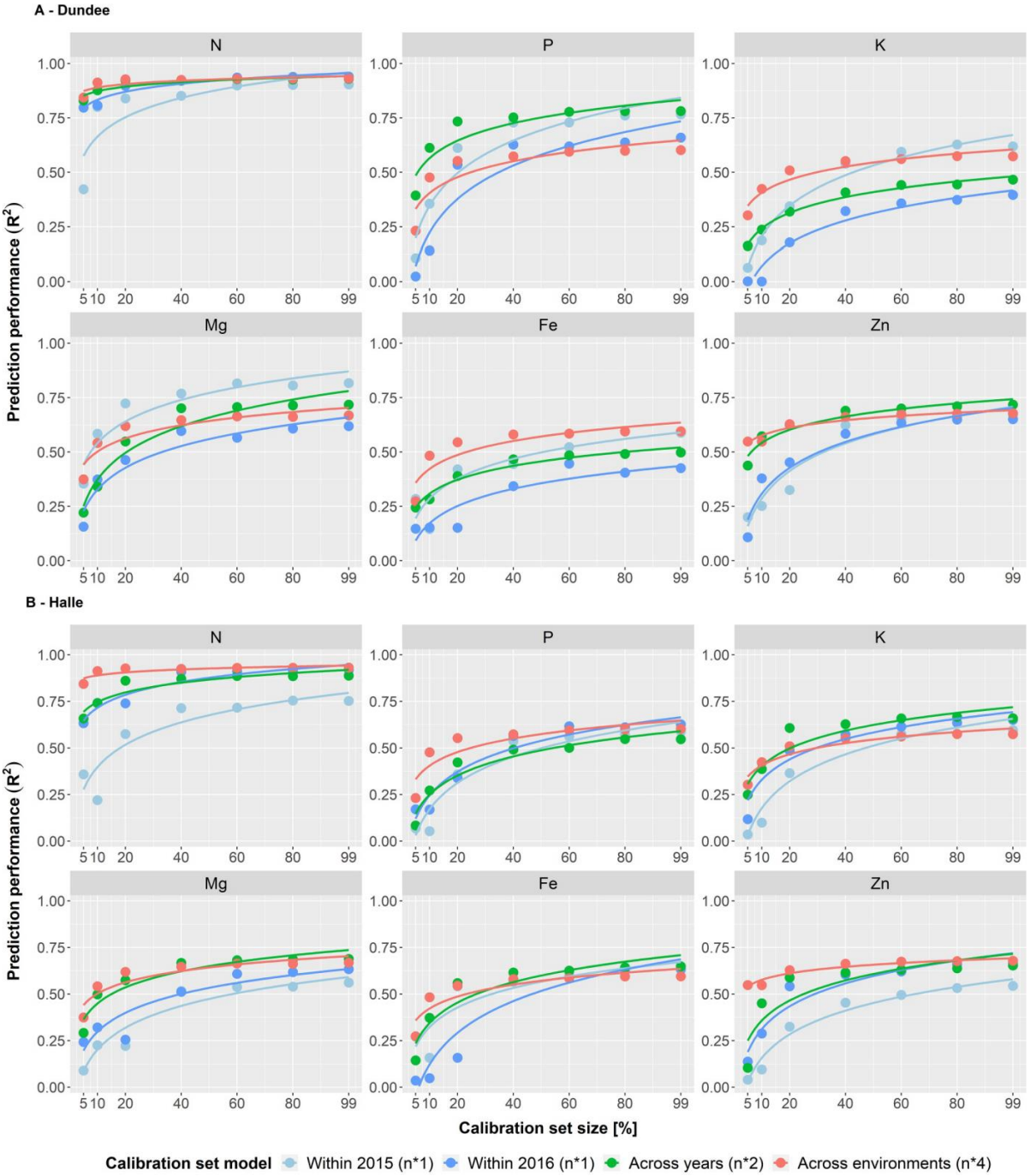

Fig 5. Calibration model comparison—With additional samples—Within environments—Within traits. Comparison of the three calibration set compositions (within environments, across years & across environments) for each of the six nutrient traits (N, P, K, Mg, Fe & Zn) in Dundee and Halle. The colors of the lines represent the different calibration set models. In addition, the legend contains the number of samples used for the respective model composition (n*1 = single number of samples, n*2 = duplicated number of samples & n*4 = quadruplicated number of samples) in parentheses.

https://doi.org/10.1371/journal.pone.0224491.g005

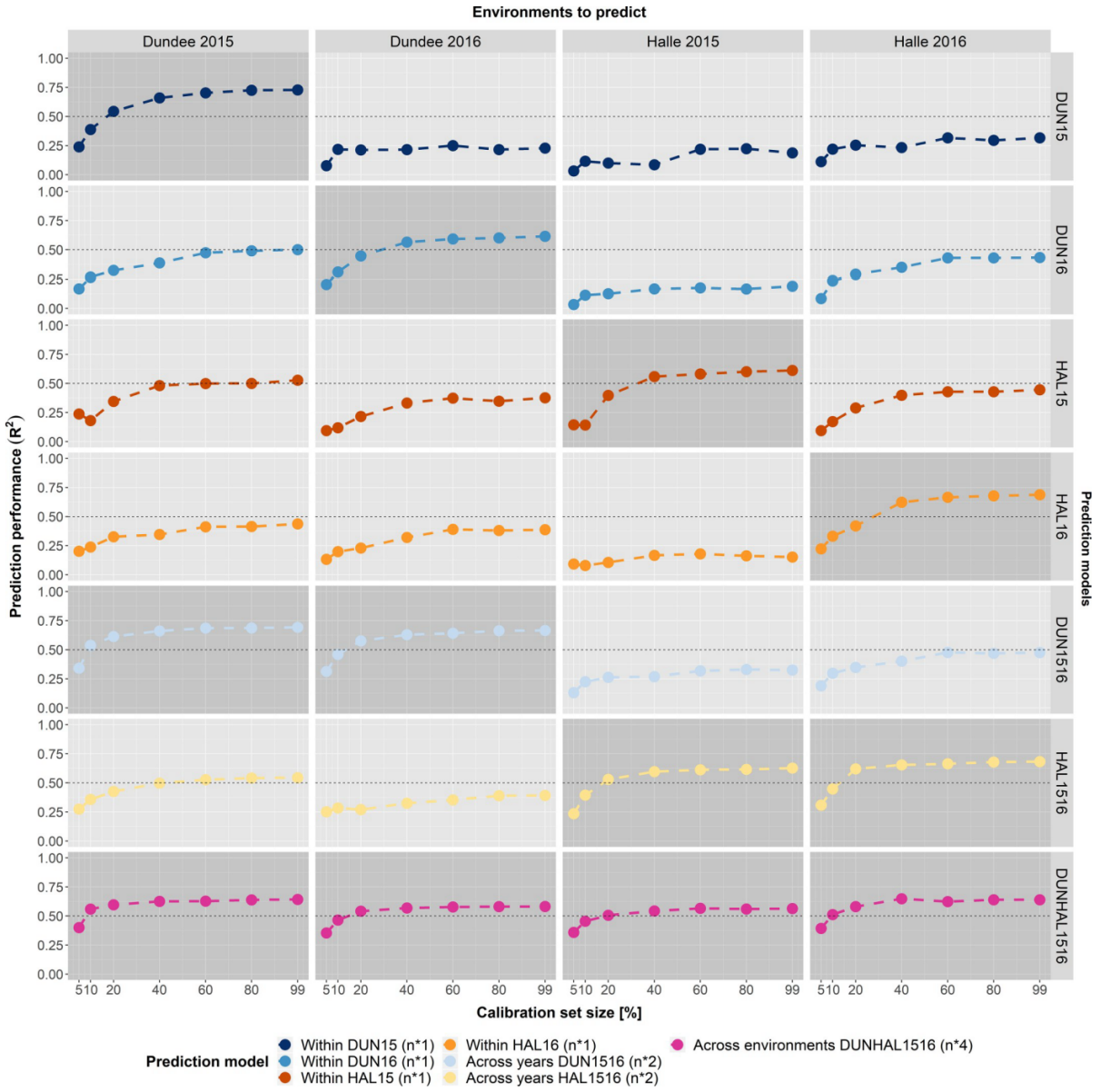

Fig 6. Model transferability—Within environments—Across traits. Evaluation of model transferability to predict grain nutrients in each of the four environments (Dundee 2015, Dundee 2016, Halle 2015 & Halle 2016, shown as columns) across the six nutrient traits (N, P, K, Mg, Fe & Zn). Seven different prediction models (within each environment, across years, across environments; shown as rows) were used to predict nutrient concentrations of the six traits in the four investigated environments. Prediction models containing the respective environment to be predicted are visually emphasized. The three types of prediction model compositions contain different numbers of samples: the four within environment models (DUN15, DUN16, HAL15 & HAL16) contain the simple number of samples of the respective environment, the two across years models (DUN1516 & HAL1516) the duplicated number of samples and the across environments model (DUNHAL1516) the quadruplicated number of samples.

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environments and years, the model performance is weakened. For a more robust model, more years and locations should be considered to increase the probability that similar environments are learnt with the calibration dataset. Other studies already pinpointed the expected

complexity of a purely data driven approach [26,79,81]. Moreover, as we only investigated one single highly diverse population, we cannot answer the question whether the results also hold true for other less diverse populations and whether trans-populational prediction would be possible.

Finally, a suggestion for users should be to analyze a relatively small number of samples in each location over several years to keep the cost for wet chemistry as low as possible while benefitting from the additional variation introduced through different locations and years into the calibration model. The presented results indicate that the across environments approach outperforms models within a single environment, especially if the sample number of calibration models is low (Fig 4). However, the quality of HSI predictions is excelled by classical laboratory methods [40], which might be acceptable in specific situations. For instance, modern breeding programs consist of thousands of individual genotypes, especially in early generations, where frequently a negative selection is applied to separate the wheat from the chaff. The superior speed of HSI allows breeders to obtain quality-related data already in those early generations, which would be unaffordable with wet chemistry methods.

Conclusions

Hyperspectral imaging offers users the possibility to analyze their samples in high throughput for a wide range of issues like soil composition and food safety [28,82]. Nevertheless, every spectral-based technology measures only a unique spectrum of a sample to correlate it to the investigated trait (e.g. protein content) based on a calibration model. The importance of these models is frequently underestimated as mentioned by Esteve Agelet and Hurburgh [52]. In the present study we evaluated different model design parameters and could provide information about the optimal model design, exemplified for nutrient content in mature barley grains.

In the dataset presented in this study, a linear regression model based on partial least squares (PLS, [43]) outperformed complex models based on neural networks, since it offered the best prediction performance while minimizing computational demand. Furthermore, we observed a positive relationship (mean R^2 of 0.96 in a logarithmic regression) between calibration set size and prediction performance with a local optimum at a calibration set size of 160 samples, representing 40% of the data investigated in this study. Above this point further increments in calibration set size are dispensable, since they seem to add no more variability to the calibration model. Models obtained in a certain environment were only to a limited extent transferable to other environments, considering the scope of this study. Extending those models with additional samples from other environments considerably improved the calibration performance. Models should be successively upgraded with new calibration data to enable a reliable prediction of the desired traits in future studies and practical applications of hyperspectral imaging systems, for instance in future plant breeding concepts. Furthermore, model transfer strategies should be investigated to transfer models to unknown environments.

Supporting information

S1 Table. List of scored traits.
(XLSX)

S2 Table. Descriptive statistics—Wet chemistry.
(XLSX)

S3 Table. Raw data.
(XLSX)

S4 Table. ANOVA—Regression model comparison.
(XLSX)

S5 Table. Correlations and R^2.
(XLSX)

S6 Table. Cost benefit analysis—Additional samples—Delta.
(XLSX)

S7 Table. Calibration model comparison—ANOVA & Tukey.
(XLSX)

S8 Table. Descriptive statistics—HSI.
(XLSX)

S9 Table. Model transferability R^2.
(XLSX)

S1 Fig. Hyperspectral imaging laboratory rack.
(PDF)

S2 Fig. Regression model comparison—Dundee 2015—Across traits.
(PDF)

S3 Fig. Regression model comparison—Dundee 2016—Across traits.
(PDF)

S4 Fig. Regression model comparison—Halle 2015—Across traits.
(PDF)

S5 Fig. Regression model comparison—Halle 2016—Across traits.
(PDF)

S6 Fig. Regression model comparison—Across environments—Within traits.
(PDF)

S7 Fig. Calibration set size comparison—Dundee 2015—Within traits.
(PDF)

S8 Fig. Calibration set size comparison—Dundee 2016—Within traits.
(PDF)

S9 Fig. Calibration set size comparison—Halle 2015—Within traits.
(PDF)

S10 Fig. Calibration set size comparison—Halle 2016—Within traits.
(PDF)

S11 Fig. Cost benefit analysis—With additional samples—Within environments—Within traits.
(PDF)

S12 Fig. Cost benefit analysis—With additional samples—Within environments—Across traits.
(PDF)

S13 Fig. Cost benefit analysis—With additional samples—Across environments—Within traits.
(PDF)

S14 Fig. Relationship between trait value range covered by the calibration set and prediction performance (R^2)—Across environments—Within traits.
(PDF)

S15 Fig. Model transferability—Within environments—Within traits.
(PDF)

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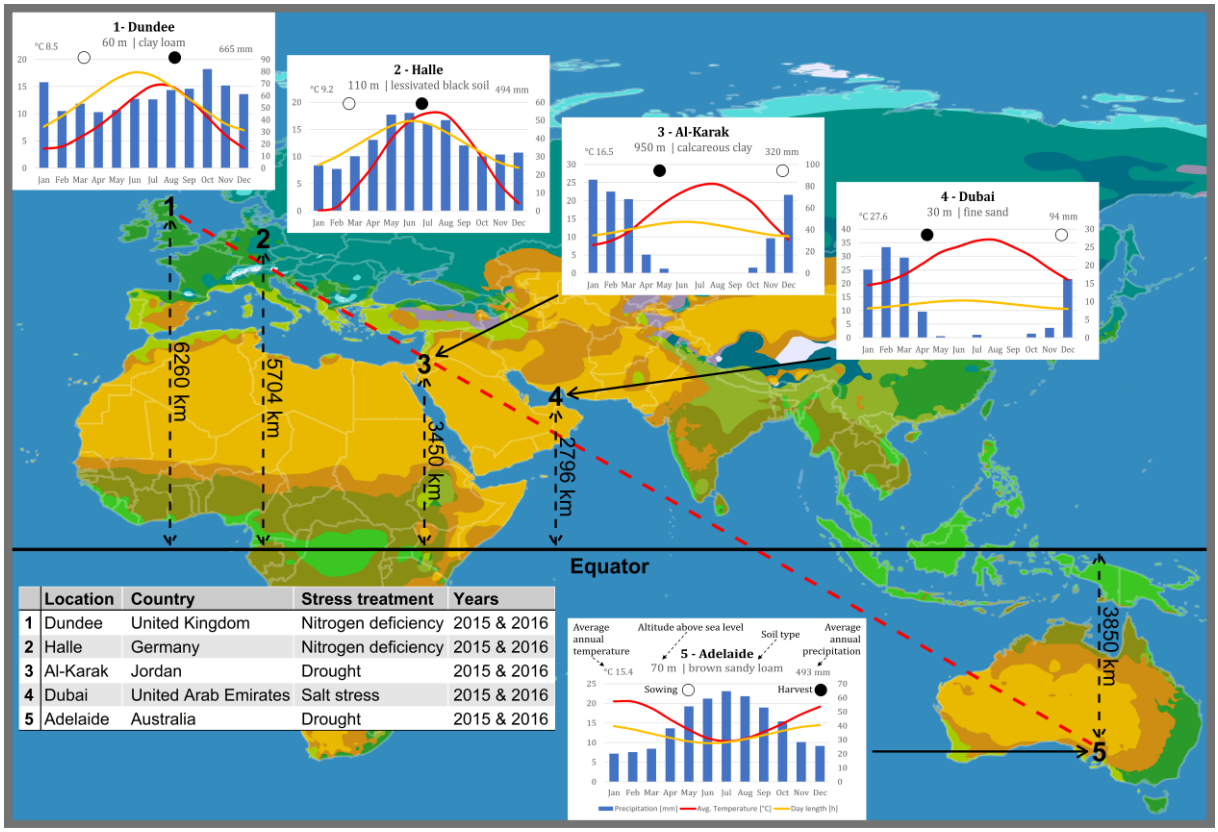
III. General discussion

III.I. Can we construct the perfect barley cultivar through timing of plant development ?

From a plant physiologist's perspective, the life of a plant begins with seed germination in a suitable environment, which could be a fruitful and wet soil or the branch of a tropic tree (Fitter and Hay 2002; Goss 2014). The manifold forms and life strategies of plants account for a multiplicity of different developmental stages, which they are passing through their life cycle (Fitter and Hay 2002; Goss 2014) to enable and increase the success of reproduction and gene dispersion as an ultimate goal. Finally, for all annual plants the cycle ends with their death, as well as the production of mature and fertile seeds, enabling to start a new growth cycle in the next season (Mauseth 2014).

By changing the view to a breeder and farmer, reproduction of a crop plant is still important. Nonetheless, it is the quantity and quality of seeds, defining the final evaluation of a crop's life cycle (Fageria 2006; Pessarakli 2014). Quantity (grain yield) and quality (grain quality) are the products of a number of traits established during the phases of plant development (Fig. 6; Slafer 2003; Sreenivasulu and Schnurbusch 2012), whereupon flowering time is probably the most critical, since plants switch from vegetative to generative growth (Worland 1996; Cockram et al. 2007; Kamran et al. 2014).

Previous studies already demonstrated that flowering time regulator genes exerted impacts on plant development throughout the whole growing period (Huijser and Schmid 2011; Maurer et al. 2016; Herzig et al. 2018). This statement is in agreement to findings of a worldwide field study (Wiegmann et al. 2019a), where four major flowering time genes in a wild barley population in a diverse set of environments were investigated (Fig. 11).



	Location	Country	Stress treatment	Years
1	Dundee	United Kingdom	Nitrogen deficiency	2015 & 2016
2	Halle	Germany	Nitrogen deficiency	2015 & 2016
3	Al-Karak	Jordan	Drought	2015 & 2016
4	Dubai	United Arab Emirates	Salt stress	2015 & 2016
5	Adelaide	Australia	Drought	2015 & 2016

Figure 11 | Global macroclimate map with information on the five experimental locations

The position of the five (1-5) test locations are indicated on a simplified map of the Köppen-Geiger climate classification system. General information about the test locations are given in the table on the lower left-hand side including the nearest town, country, stress treatment and the years of field trials. Insets next to map positions depict long-term climate information for each test location. The average monthly precipitation in millimeters (blue bars), the average monthly temperature in degrees Celsius (red line) and the course of the day length during the year in hours (yellow line) are displayed. In addition, the general sowing and harvesting dates are indicated with empty and filled circles, respectively. The Adelaide inset on the right-hand side serves as a legend for the insets. Map source: http://en.wikipedia.org/wiki/World_map (Wiegmann et al. 2019a).

This is further supported by constant effects of the candidate genes on shooting, flowering and maturity, as well as high positive correlations between these life history traits (Wiegmann et al. 2019a). What means that early and late genotypes stay early or late until the end of their life cycle, respectively, which offers two possibilities: On the one hand, to use early developmental stages as an estimate for final maturity (indirect selection) and on the other hand, to classify lines into maturity groups (e.g. early, moderate-early, …). This concept is commonly used in maize breeding, enabling an easier cultivar choice to exploit the specific agro-ecologies of a specific environment (Bennetzen 2009). However, it should be mentioned that such a classification system is unusual for barley and wheat, since both crops offer a smaller variation regarding flowering time and growing period length than maize.

Furthermore, flowering time genes do not only regulate the occurrence of a developmental stage by accelerating or decelerating it, they also simultaneously stretch

or trim the growing period. By disregarding the influence of environmental cues, including diseases and pathogens, late maturing lines usually outperform early ones, because lateness is associated with more time to produce high yields (Cockram et al. 2007; Sacks and Kucharik 2011; Alvarez Prado et al. 2017; Wiegmann et al. 2019a). Since yield improvements can be achieved through increasing the grain number per area or grain size, as well as the harvest index (Hay 1995; Slafer 2003; Slafer et al. 2014), late lines can exploit the extended growing period to establish higher biomasses (source) to improve a single or all yield components (sink) to boost grain yield. One strategy to raise the biomass accumulation would be to extend the shoot elongation phase, giving a crop more time for leaf growth and determining the grain number per area (Fischer 1985; Sreenivasulu and Schnurbusch 2012; Alqudah and Schnurbusch 2015), as indicated by a positive correlation between this phase and yield (Wiegmann et al. 2019a). The usefulness of fine tuning the occurrence and length of plant development (sub-)phases has also been documented by Sreenivasulu and Schnurbusch (2012) who investigated the development phases responsible for the number of grains and by Maurer et al. (2016) for grain size through extending the grain filling phase. However, it is difficult to make general conclusions with respect to which phase should be extended and which should be shortened, since a recently conducted field study pinpointed to the general importance of plant development on yield and yield components, but in a distinct environment-specific manner (Wiegmann et al. 2019a).

In the same way, also yield formation showed an environment-specific scheme regarding the weighting of yield components, although grains per ear was the most prominent one independent of the environment (Wiegmann et al. 2019a), which is in agreement to previous agronomic studies (Fischer 1985; Cossani et al. 2009; Slafer et al. 2014). Based on this finding a hasty conclusion would be to breed lines with a distinct focus on grain number per area. Such an approach would be a dead end, since all yield components are highly negatively correlated; by focusing on a single yield component all other would drop (Slafer 2003; Slafer et al. 2014). Additionally, it would also limit the main yield formation to only a short time frame, since each yield component is established during a specific developmental stage (Slafer 2003). Furthermore, if the main yield formation is condensed to a short time frame the occurrence of adverse weather conditions would heavily impact yield. This is supported by observation from Wiegmann et al. (2019a) that genotypes with a pronounced single yield component are marked by low yields across several environments.

No doubt, a good crop cultivar should be characterized by yield, yield and yield! Following this exaggerated statement, we would end with genotypes having high energetic/caloric values, but with low nutritional quality (Simmonds 1995; Oury et al. 2003; Fan et al. 2008), since yield and grain ingredients (e.g. proteins and nutrients) are in the majority of cases strongly negatively correlated (Murphy et al. 2008; Acreche and Slafer 2009; Guttieri et al. 2015). This context is described as "dilution effect" (Kibite and Evans 1984; Arnon 1992) and seems to be also valid for barley (Wiegmann et al. 2019c). The dilution of grain nutrients is problematic, because they define the quality of human (Grusak and DellaPenna 1999; White and Broadley 2009) and animal food (Georgievskii et al. 1982; Suttle 2010) and are, in addition, needed by plants itself, as they contribute to various plant processes (Clarkson and Hanson 1980; White and Brown 2010; Dimkpa and Bindraban 2016). Grain yield and ingredient concentration are clearly negatively correlated, however yield and ingredient yield, i.e. the product of grain yield and ingredient concentration (Khan et al. 2018), are exclusively positively correlated (Wiegmann et al. 2019c), which fits to the well-known relationship between protein concentration and protein yield (Simmonds 1995; Simmonds 1996; Acreche and Slafer 2009; Ingvordsen et al. 2016). This offers the opportunity to improve the amount of generated nutrients by crops through keeping the breeding focus on yield, although it should be mentioned that this would reduce the quality of foods produced from such crops (Fan et al. 2008).

Furthermore, the impacts of plant development on grain nutrients are displayed by the effects of plant development on yield. For instance, if late maturing genotypes are positively associated with high yield, plant development is negatively correlated with ingredient concentration, but positively with ingredient yield (Wiegmann et al. 2019c).

Now how should a perfect crop plant look like? Of course late maturing, with an extended shoot elongation phase to establish an adequate biomass to supply high yields in the end, through a slight focus on the yield component number of grains per area, but with the drawback of grains with a lower nutritional value. Or should a perfect crop be characterized by high protein and mineral concentrations, as well as the connected handicaps by focusing on grain quality? The combination of yield and quality into a single genotype has always been a challenge (Kibite and Evans 1984; Olson et al. 1987) until today, whereupon modern approaches are summarized by the term "biofortification" (Zhao and McGrath 2009; Carvalho and Vasconcelos 2013). It should be stated that two recently published studies showed the benefit of genetic engineering technologies to

increase the Fe, Mn and Zn content in mature grains, through the use of endosperm specific transporters without negative impacts on growth and yield (Connorton et al. 2017; Menguer et al. 2018).

III.II. Is adaptation the answer ?

Independent of whether quantity, quality or both are preferred, so far the impacts of environmental cues were ignored, to find an answer to the question how an ideal plant should look like. The simple term environment compromises a multiplicity of factors like soil, climate, biotic and abiotic stresses, as well as agricultural practices, that are all influencing the formation of the trait values of a plant (Fox and Rosielle 1982; Acquaah 2012; Xu 2016). The environmental impact on crop performance is even more complicated, since each genotype shows a specific interaction with the respective environment (referred to as genotype by environment interaction, GxE), which can considerably hamper the identification and selection of superior individuals (Hill 1975; Basford and Cooper 1998). On the other hand, GxE can be seen as a measurement for the adaptive ability of a plant (Cooper and DeLacy 1994; Basford and Cooper 1998; Hereford 2009).

Regarding GxE, the views of plant physiologists and breeders differ widely from each other. While physiologists ignore or associate GxE with positive aspects regarding adaptation, breeders clearly try to reduce its extent by conducting multi-environmental trials (METs) to ultimately select genotypes exhibiting low GxE interaction (Hill 1975; Cooper and DeLacy 1994; Bernardo 2010). Independent of the different goals and approaches of plant physiologist and breeders, the latter can significantly benefit by including plant physiology knowledge into their breeding programs (Jackson et al. 1996), which has already been verified in multiple surveys (Yin et al. 2004; Fischer 2011; Mir et al. 2012), including yield (Slafer 2003). The field sites in METs should reflect the target environment as good as possible, thereby enabling a more trustworthy heritability estimation and increasing the selection gain (Cooper et al. 1993; Bernardo 2010).

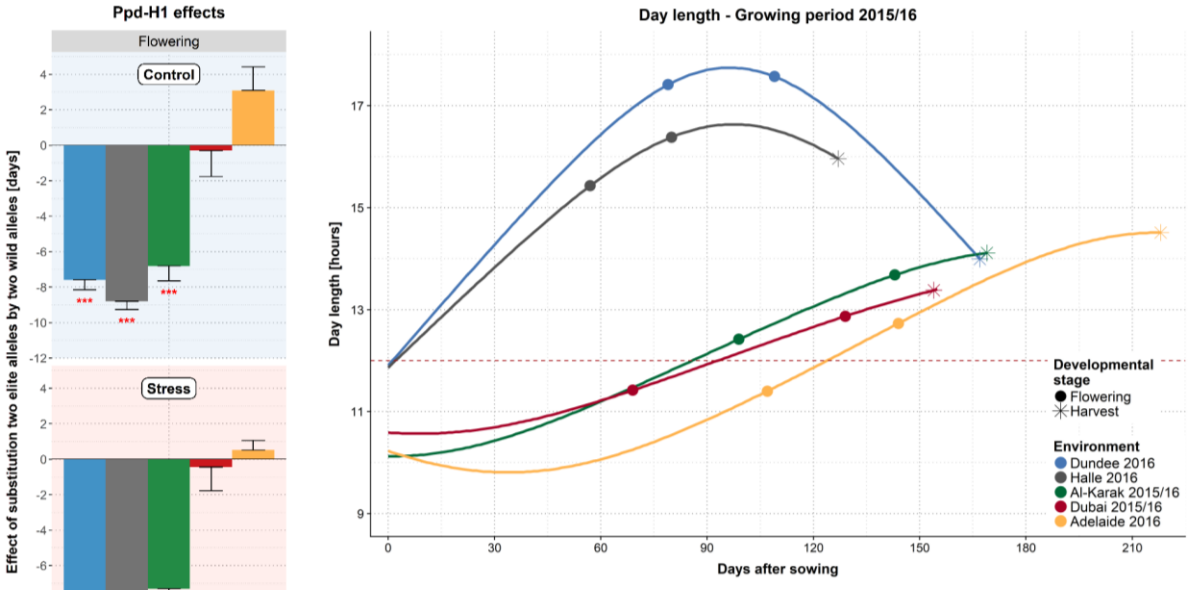
Recently, conducted field studies in barley with diverse test sites, ranging from Scotland on the northern hemisphere to South Australia on the Southern Hemisphere, confirmed the existence of pronounced GxE for developmental, yield-related and ingredient traits (Wiegmann et al. 2019a; Wiegmann et al. 2019c). In this case, one option to circumvent

the drawbacks of GxE or rather to benefit from them is to subdivide the environments into smaller breeding regions (Fox and Rosielle 1982), which reflects the strategy of local adaptation or also called decentralized selection (Simmonds 1991; Ceccarelli et al. 2000). The existence of environment specific trait formations and gene effects support the necessity for local adaptation (Wiegmann et al. 2019a). Especially genes controlling plant development (e.g. *Ppd-H1*, *Vrn-H1*, *Sdw1*) are suitable tools to adapt crops to the their target environment (Distelfeld et al. 2009; Maurer et al. 2016; Wiegmann et al. 2019a). Furthermore, as already discussed, these genes also influence yield formation and quality. In addition they are an option to avoid abiotic stresses like drought or heat periods (Kazan and Lyons 2016). For instance, if an environment is characterized by the frequent occurrence of low precipitation and/or high temperatures at the end of the growing period, the preferred breeding goal is earliness (Shakhatreh et al. 2001; Al-Ajlouni et al. 2016), which can be achieved by combining early alleles of several plant regulation genes to shorten the life cycle (Wiegmann et al. 2019a). In addition, also the opposite is possible, to exploit a prolonged growing period to accomplish high yields (Wiegmann et al. 2019a). No doubt, breeders did a great job in the past by adapting our crop cultivars to their target environment, however, the already present adverse impacts of climate change, make a re-adaption of our crops necessary (Battisti and Naylor 2009; Ceccarelli et al. 2010).

It should be mentioned that with wide adaptation a contrary approach to the above stated exists. The main difference is the definition of so called mega-environments and subsequent breeding of lines with larger geographical use (Hill et al. 1998). The International Maize and Wheat Improvement Center (CIMMYT) had considerable success with this strategy, in particular in least developed countries (Braun et al. 1996). However, well-adapted cultivars should have the potential to outperform those with wider adaptation. Furthermore, one explanation for the success of these lines might be the lack of breeding companies based in the least developed countries, which could conduct local breeding programs, like in the industrialized countries.

The importance of flowering time as a key trait for breeders and farmers is without any question, since it defines adaptation, abiotic stress tolerance and yield as shown before. The high heritability and deeply understood genetic regulation further support its usefulness in science and breeding (Jung and Müller 2009; Blümel et al. 2015; Maurer et al. 2015). Nevertheless, Wiegmann et al. (2019a) could detect a huge phenotypic variation for flowering time in wild barley. Genetically identical plants flowered on average between 66 days after sowing in the earliest site and 113 days in the latest site,

corresponding to a range of roughly one and a half months. This clearly demonstrates the tremendous impact of environmental cues on trait formation in crops, even for highly heritable traits. As already mentioned also gene effects are subject to environmental influences, for instance *Ppd-H1,* the main photoperiodism regulator in barley (Laurie et al. 1995; Turner et al. 2005). Whenever plants carry the dominant allele (sensitive form) of this gene and are grown under long day conditions (generally more than 12 hours of light) *Ppd-H1* promotes plant development, especially flowering (Turner et al. 2005; Johansson and Staiger 2015; Maurer et al. 2015). Day length is mainly defined by the time of the year and the distance of a place to the equator, whereupon places with a closer distance to the equator are characterized by more constant and shorter day length (Forsythe et al. 1995). Wiegmann et al. (2019a) observed considerable effects of *Ppd-H1* in Dundee, Halle and Al-Karak, where lines with the dominant allele exhibited an accelerated flowering time (Fig. 12).



Environment	Dundee	Halle	Al-Karak	Dubai	Adelaide
Distance to equator [km]	6260	5704	3450	2796	3818
Average temperature [°C]	11.8	13.9	10.7	22.2	13.5

Figure 12 | Influence of day length on estimates of *Ppd-H1* wild allele effects on flowering time

The left bar plot depicts the estimated *Ppd-H1* effects and the right line plot indicates the course of day length in hours during the growing period 2015/2016. The color always represents the environment, blue for Dundee, grey for Halle, green for Al-Karak, red for Dubai and yellow for Adelaide. *Ppd-H1* wild allele effects under control and stress treatments are depicted with a bright blue (top) and a bright red background (bottom), respectively. Statistically significant wild allele effects are indicated by red asterisks above or below the bars with $P < 0.05$ = *, $P < 0.01$ = ** or $P < 0.001$ = ***. The height of the bars indicates the size of the *Ppd-H1* wild allele effect, obtained by calculating the difference between the mean performance of HEB-YIELD lines carrying two wild alleles (= sensitive) versus two elite alleles. In the right plot, the time points of flowering and harvest are indicated by circle and star symbols, respectively. The first and the second appearance of the symbols specifies the first and the last occurrence of the stage across all

HEB-YIELD lines. A day length of 12 hours is indicated by a dashed red line (data of figures obtained from Wiegmann et al. 2019a).

Their results led to three notable observations: firstly, the effect size of *Ppd-H1* is similar in Dundee, Halle and Al-Karak, although the latter location is more than 2000 km closer to the equator. Secondly, by further decreasing the distance to the equator (-650 km) the effect of *Ppd-H1* disappears, as indicated by data from Dubai. Thirdly, in Adelaide, which is 350 km more distant from the equator than Al-Karak, no effect was detectable. This may be a result of the sowing time in South Australia (Fig. 12). All three findings pinpoint to the dependency of *Ppd-H1* effect sizes on day length. It can be assumed that the day length in Dundee, Halle and Al-Karak reached the required level to trigger the long day responsive effect of the dominant allele of *Ppd-H1* (Turner et al. 2005). On the contrary, in Dubai and Adelaide no or only small effects were present, probably because day length was below the threshold. By comparing day length curves between Al-Karak and Dubai it is obvious that their differences are negligible, however only Al-Karak showed an impact of *Ppd-H1* on flowering time. Therefore, it could be assumed that a threshold for the day length dependent effect of *Ppd-H1* exists, which is necessary to trigger the effect. A further increase of day length has only minor impacts on the effect size. This threshold appears to be highly specific, but the *Ppd-H1* effect sizes are not. The main reason for the absence of effects in Adelaide may be the sowing date, which is early April until end of May in South Australia and during that time day length is still falling and starts only slowly to increase in the following weeks.

If day length would be the only factor controlling the *Ppd-H1* effect size, the highest impact should have been detected in Dundee, since this environment is 550 km north of Halle and has therefore a considerable longer day length. However, Herzig et al. (2018) and Wiegmann et al. (2019a) detected the strongest effects originating from the dominant allele of *Ppd-H1* in Halle, which could be the result of a higher temperature in Halle in comparison to Dundee (Fig. 12). A previous study already reported an upregulating effect of higher temperatures on *Ppd-H1* and other members of the circadian clock (Ford et al. 2016). Furthermore, it should be noted that the regulation of flower induction is even more complex and is not restricted to day length and temperature. The induction occurs already in the period before anthesis and is therefore also controlled by environmental cues during this time (including day length) (Andrés and Coupland 2012; Alqudah and Schnurbusch 2014; Fjellheim et al. 2014).

In summary, the above mentioned examples indicate the complexity of the impact of environmental cues on multiple aspects of a plant's life cycle. This prohibits generalized conclusions without knowledge about the respective environment. Nevertheless, we may answer the question whether adaptation is the answer with yes and no:
Adaptation is not the answer for all agronomical issues; we still need a further general genetic improvement of our crops in regard to yield, quality, as well as resistance and tolerance versus biotic and abiotic stresses, respectively. Moreover, even the best climate prediction models contain errors and uncertainties how our future climates will look like (Stocker et al. 2013), which makes it challenging to breed well adapted crop cultivars. In addition, the establishment of ever-smaller local adaptation programs need higher investments in comparison to centralized breeding strategies. However, an enhanced adaptation or re-adaptation to changing climate conditions can help to close the known gap between yield potential and current farm yields (Licker et al. 2010; van Wart et al. 2013), especially under harsh environmental conditions (Lobell et al. 2009). A profound knowledge of the target environment itself is necessary, as well as estimating the magnitude of genotype specific interactions with the environment to enhance adaptation. Therefore, the interdisciplinarity of plant physiology and breeding might again be a valuable approach. However, improvements and adaptation of crops are only possible if adequate levels of genetic variation are present in the breeding pools.

III.III. Call for WILD power - overcoming our challenges ?

Genetic variability is the basis for every breeding-related activity, from crossing over selection until introgression of new alleles into the breeding pool. Breeding can be simply described as periodic alternation between the generation of variation and its subsequent reduction. This is also termed recurrent selection, since this process is a never ending story, as long as variation is present. Accordingly, without variation no breeding progress is possible (Allard 1999; Bernardo 2014). Furthermore, there exists a complex relationship between the phenotypic variation that defines the individual fitness of a genotype and the interaction between genetic and environmental variation, which are forming the phenotypic variation (Schmid 1992; Rutherford 2000).
New variability can be simply generated through the crossing of two unrelated or rather genetically different parental genotypes, whereupon the amount of generated variability

mainly depends on the genetic similarity between the two individuals (Allard 1999; Bernardo 2014). A lot of variation was lost during the millennia of natural and artificial selection. Therefore, the wild ancestors of modern crops are an undisputable useful source for acquiring new variation through the introgression of wild alleles into the elite breeding pools (Tanksley and McCouch 1997; Zamir 2001; McCouch et al. 2013; Zhang et al. 2017).

In barley, two recently released wild barley NAM populations showed the value of wild material to replenish the gene pools (Maurer et al. 2015; Maurer et al. 2016; Saade et al. 2016; Nice et al. 2016; Nice et al. 2017; Vatter et al. 2017; Herzig et al. 2018; Vatter et al. 2018). The following results are discussed based on a subset of the HEB-25 population, which is called HEB-YIELD, and compromises 48 HEB-25 lines. The main advantages of HEB-YIELD lines in comparison to the full set of HEB-25 lines is the absence of brittle rachis, the presence of an adequate threshability and the nearly equal segregation of the HEB-YIELD lines for the four selected flowering time genes, allowing to measure yield performance in field plot trials and to associate differences in plant performance with the action of these flowering genes (Wiegmann et al. 2019a). In addition, both phenotype characteristics facilitate the direct use of such lines as crossing parent in breeding programs.

In two HEB-YIELD studies high phenotypic variation of developmental, yield-related and grain ingredients traits were obtained (Wiegmann et al. 2019a; Wiegmann et al. 2019c). For instance, the plant developmental stages shooting, flowering and maturity showed across the five environments a difference between the earliest and latest HEB-YIELD line of on average 16, 28 and 22 days, respectively (Wiegmann et al. 2019a). This variation is to a high extent determined by genetics, as indicated by high heritabilities of 0.93 on average (= broad-sense heritability). Parts of the genetic variability could already be attributed to the flowering regulator genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3*. The variation for plant development might help to better adapt crops to their target environment, as well as to re-adapt them to cope with upcoming challenges of climate change. By substantially extending the growing period of barley in both directions, it might be worth to consider the introduction of a maturity group system like in maize to classify barley cultivars.

In particular for biofortification, wild material could play a more pronounced role since apart from the last century the investigation of grain ingredients like proteins, iron and zinc was impossible. Therefore, grain ingredients have never been a main breeding target

in barley and alleles responsible for grain ingredient accumulation could have been lost through numerous rounds of recurrent selection. Wiegmann et al. (2019c) found a huge variation for macronutrients and micronutrients in wild barley, especially for nitrogen (corresponds to protein concentration), calcium, sodium, iron and zinc, where the best performing HEB-YIELD lines had a more than 50 % higher ingredient concentration than "Barke", the reference parent of the population. This variability might be a useful source for biofortification and the investigation of the genetic regulation of grain ingredient accumulation.

So far, wild material has been mainly exploited for resistance and tolerance versus biotic and abiotic stresses (Hajjar and Hodgkin 2007), however there are few studies that reported on using wild progenitors for improving grain yield, like Talame et al. (2004), Nevo and Chen (2010) and Wiegmann et al. (2019a). In the latter survey wild barley lines with a superior ear number, grain number per ear or thousand grain weight than local check cultivars could be detected. These lines could be used to improve those traits in the barley breeding pool, but keeping the negative correlations of yield components in mind (Slafer 2003; Slafer et al. 2014). To get an impression of the amount of variation present in the population, 28 HEB-YIELD lines showed a more than 30 % higher ear number (per square meter) than the checks, whereupon the best one (HEB_10_173) reached more than 70 % in Al-Karak and Dubai. The higher ear or rather tiller capacity of HEB-YIELD lines, especially in Al-Karak and Dubai, might help to achieve a better soil coverage, reduced moisture losses (Ceccarelli et al. 2010; Al-Ajlouni et al. 2016) and a higher ability to suppress weeds. However, it should be stated that this trait showed the lowest heritability with an average of 0.39 and was the most laborious trait to measure. These constraints are well known, since tiller number is heavily shaped by environmental cues and shows the most pronounced plasticity of the yield components (Sadras and Slafer 2012). Therefore, tiller number traditionally has a lower priority in plant breeding and an easier and more reliable scoring (e.g. image-based technologies like phenotyping 2.0, see Głąb et al. 2015) would help to improve the breeding progress for it. In addition, there were 14 HEB-YIELD lines possessing a more than 20 % higher grain number per ear and 17 HEB-YIELD lines with a thousand grain weight more than 30 % increased, whereupon the best one was HEB_08_096 with a 49 % higher grain weight in Dubai (Wiegmann et al. 2019a). Finally, also grain yield showed considerable variability, but in the majority of cases a reduction in comparison to the local check cultivars was observed. Nevertheless, a few HEB-YIELD lines were promising like HEB_01_132, HEB_01_104, HEB_10_184,

HEB_10_173 and HEB_05_043, which performed well across the diverse trial environments. For instance, HEB_10_173 had the highest yield under drought in Al-Karak with 28.8 dt/ha, whereas the mean yield was 17.9 dt/ha. On the other side, under optimal conditions HEB_10_184 could achieve a grain yield of 74.0 dt/ha in Dundee (mean yield 55.3 dt/ha) and reached the yield level of the local check Odyssey (74,3 dt/ha). These five HEB-YIELD lines could partly be subsumed under the above mentioned term "wide adaptation", but by having a closer look it is evident that in each environment specific lines possess the highest yields (Wiegmann et al. 2019a). This finding pinpoints to the importance of environment specific adaptation and breeding of lines for local adaptation. Furthermore, HEB-YIELD lines like HEB_10_184 in Dundee, HEB_10_173 and HEB_05_043 in Al-Karak, HEB_15_082 in Dubai or HEB_01_132 in Adelaide could be directly used as parents in crossings.

In addition to the above mentioned variation for plant development, quality, yield components and grain yield, the HEB-YIELD lines could also carry new alleles to improve resistance against plant diseases, like net blotch (*Pyrenophora teres* f. *teres*), stripe rust (*Puccinia striiformis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*), as shown by surveys from Vatter et al. (2017) and (2018) in HEB-25.

The above stated examples clearly support the usefulness of wild barley material to replenish the barley elite gene pool with new variation, but it should also be mentioned that the use of wild material has some substantial drawbacks. By introgressing specific alleles or genomic regions from wild progenitors into elite genotypes, it is nearly impossible to transfer only the gene(s) of interest, because of genetic linkage between the targeted loci and undesirable genes, which is also described as "linkage drag" (Feuillet et al. 2008). The introgression of genes is traditionally achieved through an initial cross between the wild and elite genotype and several subsequent rounds of backcrossing to restore the elite background as far as possible. Depending on the length of a crop's life cycle, these steps may require several years. For breeders time is an essential factor, even if the term is not included in the response to selection formula (see below; Allard 1999), which is also stated as *"probably the most valuable tool provided to the plant breeder by statistical geneticists"* (Becker 2011).

$$R = i * h * \sigma_G$$

, where

R = Expected response to selection

i = intensity of selection

h = root of heritability

σ_G = root of genotypic variance

Nowadays, there are more sophisticated, but also more complex strategies for gene introgression like the agrobacterium (*Agrobacterium tumefaciens*) mediated gene transfer (Gelvin 2003) or the recently implemented CRISPR-Cas9 (Jinek et al. 2012) technology in plants, especially the last one looks promising since it may enable the selective introgression of genes without linkage drag through homologous recombination (Liu et al. 2017). The main barrier for this technology is the unclear political situation in Europe regarding deregulation of genetic engineering methods (Jones 2016), whereupon the public discussion can be compared to a witch-hunt in medieval times.

Moreover, wild is not a synonym for power or superiority. The majority of the 1,420 HEB-25 lines is unsuitable for modern agricultural practices, because of several reasons like bad threshability, seed shattering through brittle rachis, severe lodging, restricted machine harvest and reduced germination capacity, probably as a result of increased seed dormancy (Takeda and Hori 2006; Vanhala and Stam 2006; Sato et al. 2016). Additionally, not every wild genotype is holding a huge and/or the needed variation. The HEB-YIELD lines showed comparable drawbacks, although they were selected for having an adequate threshability and no brittle rachis. If a line shows a yield reduction of 50 % or even more in comparison to the average yield level, it is hardly acceptable as crossing parent in a traditional backcross strategy. On the other side, in breeding programs dozens of crosses are performed year by year resulting in hundreds of hypothetical cultivars, whereof finally only a few genotypes are able to outperform existing cultivars (Allard 1999; Bernardo 2014). In the end, it is more a question how to allocate the limited resources of a breeding program as efficient as possible to maximize the gain from selection. Therefore, the breeders need to decide if they preferably dig for new variation, summarized as pre-breeding or if they cross and test the already present variation for the identification of superior genotypes. However, one has always to keep in mind that without variation no breeding progress is possible.

III.IV. Should we wreck the lab ? – emergence of hyperspectral imaging

With the development of new analytical methods beginning in the last century, it was possible to investigate so far unknown plant characteristics, like the elemental composition of plant tissues or the baking quality of flour made from cereals. From today's view, these classical methods are well established and subsumed under the term "wet chemistry analysis" (Foley et al. 1998; Stuth et al. 2003; Spielbauer et al. 2009). These characteristics offered the establishment of new breeding goals and the improvement of quality related traits (e.g. protein concentration, falling number, digestibility). However, wet chemistry analyses are unsuitable for high throughput in modern breeding programs, where thousands of genotypes need to be phenotyped every year (Osborne 2006; Diepenbrock and Gore 2015). The emergence of spectral based technologies like hyperspectral imaging might offer the chance to overcome the limitations of the classical methods (ElMasry and Sun 2010; Park and Lu 2015).

Now, should we wreck the lab? Of course not! Every spectral based technology cannot stand alone, because the creation of its calibration model still needs wet chemistry analysis. Calibration models are necessary to quantitatively and qualitatively relate the measured spectra and phenotypic values. Furthermore, to a high extent they define the accuracy and precision of predicting phenotypic values based on spectral data (Batten 1998; Foley et al. 1998; Cen and He 2007; ElMasry and Sun 2010; Li et al. 2014). Luckily, there are possibilities to improve the validity of calibration models, like the choice of the regression model, the number of samples in the calibration set or the inclusion of samples from several years and locations in calibration sets (Esteve Agelet and Hurburgh 2010; Cao 2013), which was recently supported by a study investigating nutrients in mature grains using HSI (Fig. 13; Wiegmann et al. 2019b).

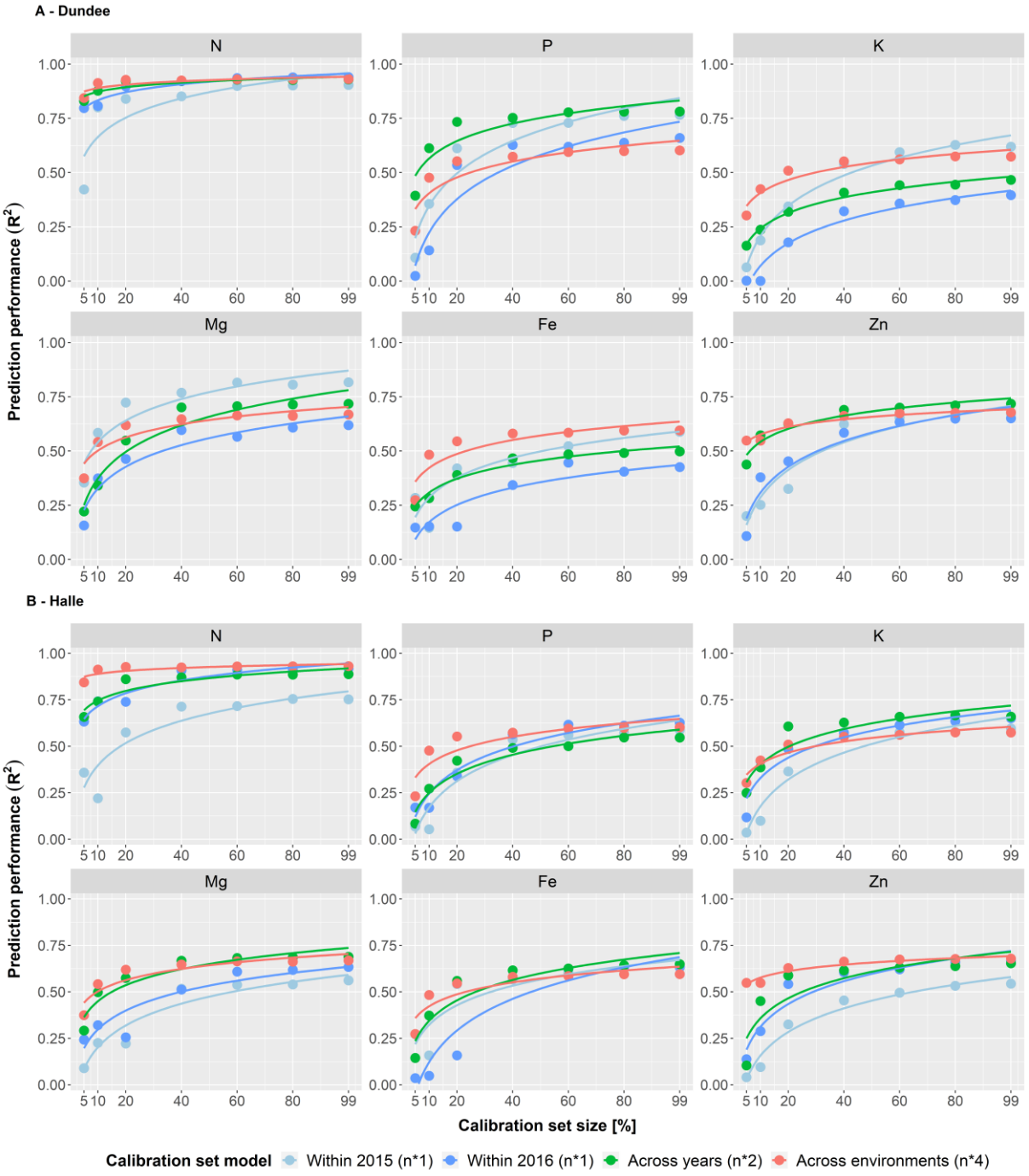


Figure 13 | Impact of different calibration set compositions on prediction performance in HSI

Comparison of the three calibration set compositions (within environments, across years & across environments) for each of the six nutrient traits (N, P, K, Mg, Fe & Zn) in Dundee and Halle. The across years model contains an equal number of samples from 2015 and 2016 and the across environments model contains an equal amount of samples from the four investigated environments (DUN15, DUN16, HAL15 & HAL16). The colors of the lines represent the different calibration set compositions. In addition, the legend contains the number of samples used for the respective model composition (n*1 = single number of samples, n*2 = duplicated number of samples & n*4 = quadrupled number of samples) in parentheses. The different sizes of calibration sets are indicated on the x-axis. On the y-axis the prediction performance is shown as the coefficient of determination (R^2) (Wiegmann et al. 2019b).

They could show that not always the most sophisticated regression and calibration model generates the best prediction performance and, additionally, that the prediction accuracy

is highly dependent on the trait, even if they seem similar (in this case nutrients). However, the most interesting finding was the indication of a relationship between phenotypic variation and prediction performance in a calibration model. The enhancement of a calibration model through additional samples, independent of whether they are originating from the same or different ones, is only useful as long as the new samples add more variation to the calibration model, leading to a general improvement of prediction performances. They suggested that for the investigated nutrients N, P, K, Mg, Fe and Zn the calibration model should consist of at least 160 samples (corresponding to a calibration set size of 40 % in Fig. 13) to allow reliable predictions (Fig. 13; Wiegmann et al. 2019b).

For N the coefficients of determination (R^2) reached a maximum level of 0.97. In contrast, even with their best performing calibration model Wiegmann et al. (2019b) could only achieve coefficients of determination between 0.50 and 0.75 for P, K, Mg, Fe and Zn, which indicates that their HSI predictions are to some degree biased and below the quality of wet chemistry based methods (Wiegmann et al. 2019c). However, the lower quality can be tolerated because of the substantial time saving. Furthermore, while in the past only breeding material from later stages has been analyzed for complex quality traits, HSI offers the possibility to already select genotypes in early breeding material where a lower precision is acceptable. However, there will always be a place for wet chemistry based analysis, since in some registration processes like malt accreditation the classical methods are prescribed by the industry or governmental authorities.

HSI, as well as other spectral based technologies, is beyond question suitable for high-throughput, but the validity of its predictions needs to be verified before using the generated data, for instance to make selections in a breeding program, because HSI will always produce predictions, independently how good or bad the calibration model is. The importance of verification and of having a trustworthy calibration model is frequently underestimated as mentioned by Esteve Agelet and Hurburgh (2010). Depending on how difficult it is to measure a trait based on its spectra the required complexity of a calibration models varies (Cao 2013; Esteve Agelet and Hurburgh 2014) and gets even more complex if the trait is affected by year and location effects (León et al. 2004; Roger et al. 2008; Shetty et al. 2012). In summary, the creation of a suitable calibration model is an additional effort, especially if the model needs to include phenotypes of several years and by taking into account that the transferability of these models is limited (Feudale et al.

2002; Cen and He 2007; Liu et al. 2014; Wiegmann et al. 2019b). Lastly, it should be stated that this effort isn't necessary if traditional wet chemistry based methods are used. Nevertheless, if the desired trait(s) can be predicted trustworthily, HSI offers a new dimension by increasing the amount of measurable samples. Moreover, HSI camera systems have been mounted already on unmanned aerial vehicles (UAV; also known as drones) to speed up on-field measurements of, for instance, weed detection (López-Granados 2011), crop management (Uto et al. 2013; Vega et al. 2015) and to capture the photosynthetic activity of plants (Zarco-Tejada et al. 2013). In regard to plant nutrients it would be interesting to use a combination of HSI and UAV to track the nutrient status of plants during the growing period and to identify genotypes with superior nutrient concentration already in the field.

In summary, the presented studies demonstrate that plant breeders have several established and new technologies at their disposal to achieve the needed yield improvements from year to year, but it should be mentioned that there are considerably more breeding goals than just yield. The difficulty is how to allocate the limited resources of a breeding program to maximize genetic gain. Wild germplasm is without any doubt a useful source to replenish the gene pools of our crops and it can be used for more than only to improve pathogen resistance and abiotic stress tolerance. Nevertheless, it is not the answer for every breeding related obstacle, especially since the steps of identifying useful variation and their subsequent incorporation into the elite breeding pools is time consuming.

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List of abbreviations

AP	Awn primordium
At	Anthesis
BGF	Begin grain filling
CIMMYT	International Maize and Wheat Improvement Center
Cl	Collar initiation
CO	Constans
CVI	Climate vulnerabilities index
DNA	Deoxyribonucleic acid
DR	Double ridge
Em	Seedling emergence
FAO	Food and Agriculture Organization of the Untited Nations
FT	Flowering locus T
GA	Gibberellic acid
GA20ox	Gibberellic acid 20 oxidase
GxE	Genotype by environment interaction
Hd	Heading time
HEB	Halle Exotic Barley
HSI	Hyperspectral imaging
Hv	Harvest
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
IPCC	Intergovernmental Panel on Climate Change
LD	Linkage disequilibrium
MAGIC	Multi-parent Advanced Generation Inter-Cross
MAS	Marker-assisted selection
MTAs	Marker trait associations
NAM	Nested Association Mapping
PM	Physiological maturity
Ppd-H1	Photoperiod-H1
QTL	Quantitative trait locus
R^2	Coefficient of determination
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
SNP	Single nucleotide polymorphism
Sw	Sowing
UAV	Unmanned aerial vehicle
Vrn-H1/VRN1	Vernalization gene H1
Vrn-H2/VRN2	Vernalization gene H2
Vrn-H3/VRN3	Vernalization gene H3

List of figures

Appendix

Declaration under oath/Eidesstattliche Erklärung

I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word and in content.

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Wernigerode, 10.11.2019 M. Wiegmann

Signature / Unterschrift

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Mit besten Grüßen

Mathias Wiegmann

Curriculum vitae

Personal Details

Name	Mathias Wiegmann
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Date of birth	05 April, 1988
Place of birth	Bünde
Citizenship	German

Education Experience & Military Service

04/2015 – Ongoing

Ph.D. thesis - Martin Luther University of Halle-Wittenberg - Chair of Plant Breeding

- Focus : Interaction between flowering time and grain yield in spring barley (*Hordeum vulgare* L.)
- Project : "Flowering time control: from natural variation to crop improvement" (DFG SPP 1530)
- Supervisors : Prof. Dr. Klaus Pillen and Dr. Andreas Maurer

10/2011 – 09/2014

Master of Science in Biology - Technical University of Munich

- Overall grade : 1.3
  - Focus : Plant breeding
- Master's thesis : Multi-Cross QTL analysis of the traits plant height, heading and falling number in bread wheat (*Triticum aestivum* L.)
  - Master's thesis grade : 1.0
- Contents i.a.: Molecular Plant Breeding, Genetic Selection Supported by Markers, Host-Parasite Interaction & Quantitative Genetics

10/2008 – 09/2011

Bachelor of Science in Biology - Technical University of Munich

- Overall grade : 2.1
  - Focus : Molecular botany
- Bachelor's thesis : Studies on the in vivo-interaction between the protein phosphatase ABI2 and prefibrillin 1a
  - Bachelor's thesis grade : 1.0
- Contents i.a. : Developmental Genetics of Plants, Genetics & Plant Physiology

01/2008 – 10/2008

Conscript at Panzerbataillon 203 - Augustdorf

09/1998 – 06/2007 **Abitur** - Gesamtschule Rödinghausen
- Equivalent to A-level
- Overall grade : 2.3
- Advanced courses : Biology and Math

Professional Experience

06/2019 – Present **Wheat Breeder** at the company RAGT 2n
- Field of action : Winter wheat breeding at the station Silstedt, Germany

05/2018 – 04/2019 **Cereal Breeder Internship** at the company InterGrain Pty Ltd
- Field of action : Spring barley breeding at the stations in Bibra Lake and Horsham, Australia
- Related activities :
  - Scoring of field and disease trials
  - Data analysis of field and glasshouse trials
  - Maintaining of the database

03/2013 – 10/2013 **Research Associate** at the company Pioneer Hi-Bred Northern Europe
- Field of action : Corn breeding at the stations in Langenbach and Eschbach, Germany
- Related activities :
  - Leading part-time employees
  - Guiding the data quality process of phenotypic traits
  - Support during pollination in nursery and isolations (F1 seed)

04/2012 – 10/2012 **Student apprentice** at the company Pioneer Hi-Bred Northern Europe
- Field of action : Corn breeding at the stations in Langenbach and Eschbach, Germany
- Related activities :
  - Sowing preparation and sowing
  - Taking notes during growing period, e.g. flowering time and lodging
  - Verification of new DH-lines via genetic markers

03/2011 – 05/2011 **Research Assistant** Chair of Botany - Technical University of Munich
- Field of action : Arabidopsis mutant screening, DNA isolation and examination of different plant organs, sterilisation and sowing of seeds
  - Focus : drought stress - abscisic acid

Publications

- **Wiegmann Mathias**, Andreas Backhaus, Udo Seiffert, William T.B. Thomas, Andrew J. Flavell, Klaus Pillen and Andreas Maurer (2019). "Optimizing the procedure of grain nutrient predictions in barley via hyperspectral imaging". PLOS ONE, https://doi.org/10.1371/journal.pone.0224491

- **Wiegmann Mathias**, Andreas Maurer, Anh Pham, Timothy J. March, Ayed Al-Abdallat, William T.B. Thomas, Hazel J. Bull, Mohammed Shahid, Jason Eglinton, Michael Baum, Andrew J. Flavell, Mark Tester and Klaus Pillen (2019). "Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues". Scientific Reports, https://doi.org/10.1038/s41598-019-42673-1

- **Wiegmann Mathias**, William T.B. Thomas, Hazel J. Bull, Andrew J. Flavell, Anette Zeyner, Edgar Peiter, Klaus Pillen and Andreas Maurer (2019). "Wild barley serves as a source for biofortification of barley grains". Plant Science, https://doi.org/10.1016/j.plantsci.2018.12.030

Conference contributions

- 03/2018, German Plant Breeding Conference 2018 in Wernigerode, Germany. Poster: Comparing the crosstalk between flowering time, stress tolerance and yield in the Halle Exotic Barley-Yield population at five locations worldwide.

- 09/2017, 18th Australian Barley Technical Symposium in Hobart, Australia. Talk: Comparing the crosstalk between flowering time and abiotic stress tolerance in the Halle Exotic Barley-Yield population at five locations worldwide

- 06/2016, 12th International Barley Genetics Symposium in Minneapolis, USA. Poster: Crosstalk between flowering time and abiotic stress tolerance in Halle Exotic Barley-YIELD (HEB-YIELD).

- 03/2016, Main Conference of the Genome Research Working Group of the German Plant Breeding Association (GPZ) in Bonn, Germany. Poster: Genetic control of flowering time in HEB-YLD, a wild barley (*Hordeum vulgare* L.) population studied world-wide.

- 09/2015, 18th Conference of the Genome Research Working Group of the German Plant Breeding Association (GPZ) in Düsseldorf, Germany. Poster: Crosstalk between flowering time and abiotic stress tolerance in yield of Halle Exotic Barley.

Skills

Language
- German - native
- English – very good

IT
- Word, Excel and Powerpoint - very good
- R - very good
- SAS - very good

Plant breeding
- Barley
- Maize
- Wheat

Wernigerode, 25.11.2020 _____

Signature