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Climate Change Effects to Plant Ecosystems - Genetic Resources for Future Barley Breeding

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Climate Change Effects to Plant Ecosystems

- Genetic Resources for Future Barley Breeding

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PREFACE

The present dissertation is submitted as the final of the PhD project ‘Climate Change Effects to Plant Ecosystems – Genetic Resources for Future Barley Breeding’ performed by Cathrine H Ingvordsen from January 2010 to end of April 2014. The research was conducted within the NordForsk-funded network ‘Sustainable primary production in a changing climate’ at the Technical University of Denmark, Department of Chemical and Biochemical Engineering in the Center of Ecology and Environmental Sustainability. The PhD project was funded approximately 1/3 each from the following sources: 1) the Nordic Council of Ministers (NordForsk), 2) the Danish Council for Independent Research (FTP) and 3) the Technical University of Denmark, Department of Chemical and Biochemical Engineering.

The PhD project was supervised by Senior scientist Rikke B Jørgensen (DTU-KT) and co-supervised by Senior scientist Teis N Mikkelsen (DTU-KT), Associate professor Michael F Lyngkjær (KU-Science) and Professor Pirjo Peltonen-Sainio (MTT, Fi).

The overall aim of the present PhD project was to investigate the effects of climate change to plant ecosystems, exemplified by the agro-ecosystem with barley cropping. Barley was chosen as the genetics of barley are well known, and modern genetic tools can be applied in barley. Three sub-aims were:

1) to secure future primary production by mining genetic resources that potentially could be exploited in breeding
2) to add basic knowledge on how plants respond to combined and extreme climate treatments
3) to supply input data for modeling in order to predict future climate change impacts on primary production.

Four manuscripts are included in this dissertation. The references and titles of the papers are:

1. Ingvordsen CH, Backes G, Lyngkjær MF, Peltonen-Sainio P, Jensen JD, Jalli M, Jahoor A, Rasmussen M, Mikkelsen, TN, Jørgensen RB. *Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions*
2. Ingvordsen CH, Backes G, Lyngkjær MF, Peltonen-Sainio P, Jahoor J, Mikkelsen TN, Jørgensen RB. *Genome-wide association study of production and stability traits in spring barley cultivated under future climate scenarios*

3. Ingvordsen CH, Gislum R, Jørgensen JR, Mikkelsen TN, Jørgensen RB. *Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 barley (*Hordeum vulgare *L.) accessions*
4. Ingvordsen CH, Lyngkjær MF, Peltonen-Sainio P, Mikkelsen TN, Jørgensen. *Effect of an extreme heatwave on 22 spring barley accessions cultivated in future climates. Tendencies in allocation of biomass, temperature priming,* CO_2*-responsiveness and stability of grain yield*

In addition to above four manuscripts, which are to be assessed in this dissertation, the research has been presented at international conferences and courses and manuscripts in preparation.

Conferences:

- Plant Abiotic Stress and Sustainable Agriculture: Translating Basic Understanding to Food Production, Taos, USA, January 2013 (*poster*)
- Pre-breeding – fishing in the gene pool, Alnarp, Sweden, June 2013 (*oral*)
- Genetic Resources for Food and Agriculture in a Changing Climate, Lillehammer, Norway, January 2014 (*oral, invited speaker*)

Courses (*poster*):

- Breeding for adaptation to climate change, Hvanneyri, Iceland, January 2011
- Pre-breeding for sustainable plant production, Röstånga Sweden, January 2012
- Metabolomics and Plant Breeding, Foggia, Italy, April, 2013

Manuscripts in preparation:

- Marker-trait associations in spring barley for leaf rust, net blotch, ramularia, scald and spot blotch detected by genome-wide association (*first author*);
- Field comparison of spring barley cultivars exposed to increased [CO_2] (*co-author*),
- Effects of the changing climate on the quality of barley seeds (*co-author*).
- Eco-efficient production of spring barley in a changed climate: a Life Cycle Assessment including primary data from future climate scenarios (*co-author*).

Roskilde, April 2014

Cathrine Heinz Ingvordsen

SUMMARY

Climate Change Effects to Plant Ecosystems – Genetic Resources for Future Barley Breeding

A growing population and a considerable increase in living standards worldwide are increasing the demand on the primary production. At the same time, climate change is projected to lower the primary production due to increases in the atmospheric concentrations of carbon dioxide ([CO_2]) and ozone ([O_3]), rising temperatures and extreme climate events such as floods, storms and heatwaves. These predictions are compounded by the projections from the Intergovernmental Panel on Climate Change, which state that the world is heading towards a worst-case climate scenario unless actions are taken collectively in the very near future.

Crop yields have stagnated since the start of this century; a trend also revealed in the cultivation of barley and wheat in the Nordic countries Denmark, Sweden, Norway and Finland, why actions are needed to develop climate resilient cultivars and secure future primary production. Within the network 'Sustainable primary production in a changing climate' 22-138 spring barley accessions have been grown in the climate phytotron RERAF under conditions mimicking climate change; 1) elevated temperature (+5 °C), [CO_2] (700 ppm) and [O_3] (100-150 ppb) as single factors, 2) elevated temperature and [CO_2] in combination and 3) a 10 day-heatwave (33 °C) around the time of flowering in addition to elevated levels of temperature and [CO_2]. The responses in grain yield, number of grains, number of ears, biomass, harvest index, grain protein concentration and stability over treatments were assessed. In addition, a genome-wide association study of recorded phenotypes and DNA-markers (from Illumina arrays) recognized novel marker-trait associations of production parameters under climate change conditions.

In a future climate scenario of elevated temperature and [CO_2] the grain yield of barley was found to decrease by 29 % and harvested grain protein by 22 %. With an additional 10 day-heatwave around flowering grain yield was decreased by 52 %, revealing sombre forecasts to the future primary production. However, vast variation was identified within the individual barley accessions, which can be introduced into cultivars to achieve climate resilience.

The results from the present dissertation have entered into manuscripts on the direct effect of climate change on barley productivity and quality as well as in life cycle assessment studies (LCA). Valuable genetic resources were identified for possible use in breeding of climate resilient cultivars and SNP-markers that link to traits favourable in changed environments. Basic knowledge of plant response to multifactor climate treatments has been added as well as data on numerous genotypes modeling the impact of climate change to future primary production have been supplied.

RESUMÈ

Klimaforandringers Effekt på Planteøkosystemer - Genetiske Ressourcer til Fremtidens Forædling af byg

Antallet af mennesker på jorden er stigende og en samtidig forhøjelse af levestandard øger efterspørgslen til primærproduktionen. Samtidig med den stigende efterspørgsel, lyder det fra IPCC, at klimaforandringerne højst sandsynligt vil forårsage en nedgang i primærproduktionen grundet stigende niveauer af temperatur, koncentrationen af kuldioxid ([CO_2]) og ozon ([O_3]) samt ekstreme vejrfænomener, der vil blive hyppigere og længere af varighed. Ydermere forværrende for forholdene er, at vi, ifølge IPCC, er på vej mod det værst tænkelige klimascenarie uden snarlig nedgang i den globale udledning af drivhusgasser.

Stagnerende afgrødeudbytter er observeret siden starten af dette århundrede og denne tendens ses også i kerneudbyttet hos byg i de nordlige lande Danmark, Sverige, Norge og Finland. Der må skrides til handling, så klimatolerante sorter kan udvikles og sikre den fremtidige primærproduktion. I regi af netværket 'Sustainable primary production in a changing climate' er 22-138 byg-accessioner blevet testet i en klimafytotron under 1) forhøjet temperature (+5 °C), [CO_2] (700 ppm) og [O_3] (100-150 ppb); 2) forhøjet temperatur og [CO_2] i kombination og 3) under en 10 dage lang hedebølge (33 °C) omkring blomstring med forudgående forhøjede niveauer af temperature og [CO_2]. Effekten er opgjort for byg generelt og ligeledes for de individuelle byg-accessioner. Derudover kunne DNA-markører associeres med de fundne produktionsparametre.

Kerneudbyttet faldt med 29 % i et fremtidsscenarie med øget temperatur og [CO_2] i kombination samtidig med en reduktion på 22 % i høstet protein fra kernen. Under en tilsvarende klima-behandling med 10 dages hedebølge omkring blomstring faldt kerneudbyttet med 52 %, hvilket understøtter den forventede nedgang i udbytte. Imidlertid var der stor variation i klimaresponsen iblandt de inkluderede byg-accessioner. Denne variation bør udnyttes i udviklingen af klimatolerante sorter, der kan sikre fremtidens primær produktion.

Resultaterne fra klimaeksperimenterne med byg-accessionerne er blevet formidlet i manuskripter om klimaforandringernes direkte effekt på primærproduktionen og kvalitet samt i livscyklusvurderinger (LCA). Ydermere er genetiske ressourcer, der vil kunne anvendes i forædlingen af fremtidens klimatolerante sorter blevet identificeret, SNP-markører, der kobler til egenskaber af værdi i et foranderligt klima, er blevet bestemt, og grundlæggende viden er frembragt om respons hos byg på multifaktorielle klimaforsøg. De tilvejebragte data kan desuden indgå i modelstudier om klimaforandringernes indflydelse på den fremtidige primærproduktion.

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BACKGROUND

Plants are the fundament for the survival of every species that ingests food. Worldwide, crop plants are the basis of the production of food for human consumption and feed for livestock. Plants are sessile, why their growth environments are decisive for development and the final yield, which constitutes the primary production. Hence, changes in climate impact the primary production.

Climate Change

At the end of the 19th century Svante Arrhenius built on to the work by Joseph Fourier, John Tyndall and Samuel Langley and made the first calculations suggesting sensitivity of the Earth's surface temperature to changes in atmospheric carbon dioxide concentrations ([CO_2]). Arrhenius used the term 'hothouse' starting what today is known as the greenhouse effect. That the increase in the greenhouse effect was anthropogenic was first reported in the mid-20th century by Guy Callendar (Fleming, 1998). In 1988 the Intergovernmental Panel on Climate Change (IPCC) was established to summarize experimental studies to assess climate change status, potential impacts and options for adaptation and mitigation to the induced climate change. Today, the assessment reports from IPCC are the product of working groups (WG) consisting of several scientists, and the reports are the fundaments in decisions by governments, authorities, political organisations and other professional bodies.

In the most recent IPCC assessment report (AR5) atmospheric concentrations of the greenhouse gasses CO_2, ozone ([O_3]), methane and nitrous-oxide are predicted to increase together with the global mean temperature (Collins et al., 2013). The level of increase in climatic factors is dependent on the anthropogenic radiative forcing; the anthropogenic energy emissions influencing the energy system of the earth (Fig 1). The four different scenarios of anthropogenic radiative forcing (Fig 1) termed the representative concentration pathways (RCP) in AR5 and previous SCRES (Special Report on Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation) will lead to different levels of increase in temperature, greenhouse gasses and climate events. In the forecasting (modelling) is mitigation and adaptation also included. According to IPCC AR5, we are currently approaching the worst-case scenario termed RCP8.5 unless actions are taken collectively in the very near future (IPCC, 2014a). Projections are that [CO_2] in the RCP8.5 scenario will reach around 1,000 ppm at the end of the 21st century and global mean temperature will increase approximately 5 °C (Fig 1; Collins et al., 2013). Ozone is highly reactive and future levels can be decreased by elevated temperature, however, concerted increase in methane is predicted to increase [O_3] by 25 % in average according to RCP8.5 (Collins et al., 2013). In addition, to the yearly increase of temperature, [CO_2] and [O_3] intra-seasonal extreme weather events e.g. heatwave, floods and storms are predicted to increase in frequency, length and intensity (Collins et al., 2013).

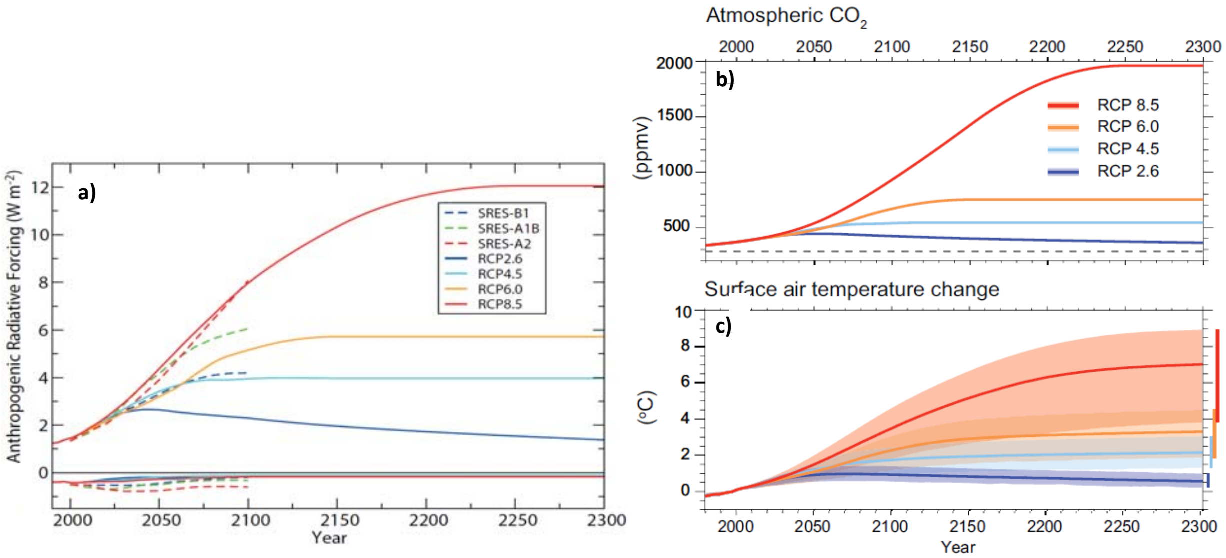

Figure 1. (a) Time evolution of the total anthropogenic (positive) and anthropogenic aerosol (negative) radiative forcing relative to pre-industrial (about year 1765) between year 2000 and 2300 according to RCP (representative concentration pathways) and SCRES **(**Special Report on Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation). Projected global atmospheric [CO_2] (**b**) and surface air temperature change (**c**) in the four RCP scenarios of IPCC AR5. Modified from IPCC AR5 WG I final report, figure 12.3 & 12.42 (Collins et al., 2013).

Generally, climate predictions are more uncertain for smaller regions than for global, however, the Nordic countries constitute a niche, since temperature changes here are added to a low level of basic temperatures. In Finland, an increase in temperature causing longer growing season has even been speculated to be advantageous for crop production (Peltonen-sainio et al., 2009). In the Nordic area temperature is predicted to rise most in the winter months (IPCC, 2007). Denmark and southern Sweden are predicted to experience temperature increases similar to the global mean, whereas temperature in northern Sweden, Norway and Finland is projected to increases close to double of the global mean. Hence, if actions are taken to bring down greenhouse gas emissions, and as a result temperature increase is below the coveted +2 °C, as recommended by IPCC (RCP4.5; IPCC, 2014a), Helsinki, Finland will hold temperatures as known today from Porto, Portugal (average temperature in July Helsinki: 16 °C and Porto: 20 °C, DMI, 2014); Helsinki and Porto differing 21 ° in latitude. Precipitation is expected to increase in the entire Nordic region during winter, however, in Denmark and southern Sweden summer precipitation may decrease (IPCC, 2007).

Climate Change Effect to Primary Production

That primary production is greatly challenged under the worst-case RCP8.5 scenario is inarguable (Schade and Pimentel, 2009; IPCC, 2014b). From a +5 °C, yields of wheat are expected to decrease 45 % in the tropical regions and 15 % in the temperate regions (Challinor et al., 2014). Numerous experiments have reported elevated temperature to decrease grain yield (e.g. Conroy et al., 1994; Clausen et al., 2011), as was also observed in the field data from 1980 to 2008 (Lobell et al., 2011), however, the responses causing decreased grain yield are complex and numerous from impairment of anthesis to perturbation of photosynthesis (Table 1; Barnabás et al., 2008). The decreased grain yield caused by elevated temperature is found to be counteracted by elevated [CO_2] increasing grain yield from increased photosynthesis and improved leaf water status (Table 1; Jablonski et al., 2002). The degree of ameliorated grain yield from elevated [CO_2] is though ambiguous (Long et al., 2006; Tubiello et al., 2007; Bloom et al., 2014). Generally, the effect of elevated [O_3] is found to decrease grain yield e.g. by reactive oxygen species interference (Ainsworth et al., 2012) with differences in O_3-tolerance between species (Mills et al., 2007). The reactivity and uneven distribution of [O_3] differ from the constant increase of [CO_2], why the effect of elevated [O_3] to the future primary production range from low to high importance.

Several of the climatic parameters affect crop performances by interfering with photosynthesis (Table 1). Considering the major role photosynthesis play in plant performance this is expected. According to Richards (2000), increase in yield has been achieved by increased or extended photosynthesis per unit leaf area and by increased partitioning of biomass in the grain filling process; hence not directly from the improvement of photosynthesis, but from plant architecture e.g. leaf size and other processes tightly linked to photosynthesis. Where the effect of single climatic factors is well studied (Table 1) their combined effect - constituting a more realistic future climate scenario - on grain yield is lagging behind (Mittler, 2006; Atkinson and Urwin, 2012). The available studies including elevated temperature together with [CO_2] commonly report decreased grain yield despite the increasing effect of elevated [CO_2] (Batts et al., 1998; Amthor, 2001; Clausen et al., 2011; Alemayehu et al., 2013). Studies of elevated [CO_2] and [O_3] in combination also reported the increased grain yield by elevated [CO_2] to be suppressed resulting in overall decreased grain yield (Fuhrer, 2003; Long et al., 2005). To our knowledge, only one study included elevated temperature, [CO_2] and [O_3] in combination and found a 40 % decrease in seed yield of oilseed rape (Frenck et al., 2011).

Table 1. Single-factor effects of elevated temperature, [CO_2] and [O_3] on C3 cereals as they are often reported. Elevated temperature is considered at over-optimal levels.

Elevated temperature	**Elevated [CO_2]**	**Elevated [O_3]**
Productivity & Development		
▪ decreased grain yield	▪ increased grain yield	▪ decreased grain yield
▪ decreased grains per ear	▪ increased grains per ear	▪ decreased grains per ear
▪ rapid growth, shortened lifecycle	▪ increased aboveground vegetative biomass	▪ decreased aboveground vegetative biomass
▪ increased grain protein concentration	▪ decreased grain protein concentration	▪ increased grain protein concentration
▪ restricted plant growth		▪ accelerated leaf senescence
▪ impaired anthesis		
▪ increased mortality		
Physiology		
▪ decreased leaf area	▪ increased leaf area	▪ decreased stomatal conductance
▪ increased stomatal water loss	▪ decreased stomatal water loss	▪ higher rates of mitochondrial respiration
▪ imbalance of photosynthesis and respiration	▪ higher net uptake of CO_2	▪ metabolic costs of detoxification
▪ reduces the photochemical efficiency of photosystem II	▪ decline in photorespiration response	▪ physiological spots
▪ changes in the organization of cellular structures		▪ triggers pathways for wounding and pathogen defence
Molecular & Biochemistry		
▪ decreased RUBISCO activity	▪ activating RUBISCO (short term)	▪ decreased RUBISCO activity
▪ misfolding and denaturation of proteins	▪ downregulating amount of RUBISCO (adaptation)	▪ decreased chlorophyll concentration
▪ transcription of heat shock proteins	▪ transcription of photosynthetic proteins	▪ activating free radicals, reactive oxygen species
▪ production of phyto-hormones (ABA) and antioxidants		▪ structural damage of membrane proteins
(Ferris et al., 1998; Barnabás et al., 2008)	(Amthor, 2001; Ainsworth and Rogers, 2007)	(Fuhrer, 2003; Feng et al., 2008; Ainsworth et al., 2012)

Following the prediction of increased frequency of extreme events and their possible strong detrimental effect on grain yield (Gourdji et al., 2013; Reyer et al., 2013), extreme events have come in focus. Experimental studies have reported effects of heatwaves on primary production, under ambient climate conditions supporting the projected decreases in grain yield (Bencze et al., 2004; Dias de Oliveira et al., 2013). Within seasons extremes can though also benefit plant production, as a short period of elevated temperature was found to mitigate the following exposure to high temperature (Barnabás et al., 2008). Hence timing in plant development and frequency of climate extremes are crucial with regard to the effects of variation on crop performance.

Investigations of climate change effect on the quality of the grain yield are progressing (Wang and Frei, 2011). In the context of protein, temperature and [O_3] as single-factors have been found to increase protein concentration, whereas elevated [CO_2] decreased the concentration possible due to dilution from increased/decreased production of starch (Savin and Nicolas, 1996; Högy and Fangmeier, 2008; Pleijel and Uddling, 2012). Few studies have reported the effect from combined climatic factors on protein concentration. One available study, reporting the effect of elevated [CO_2] and heatwave during grain filling on three cultivars of wheat, identified the three possible responses; increased, maintained and decreased protein concentration (Bencze et al., 2004). A study in barley on the influence of elevated temperature (soil temperature +2 °C) together with reduced precipitation (42 mm less than ambient) no change on protein concentration was reported, but in the protein composition (Högy et al., 2013). Despite preceding research in grain quality under multi-factor treatments, knowledge-gaps unarguable exist (Dupont and Altenbach, 2003).

Studies of single-factors are more numerous than studies with combined climatic factors, with the consequence that simulation studies, predicting the primary production of the future, build on the response to single-factor studies, why considerable uncertainty is embedded in the predictions. However, from recent years' real life data there is little doubt that primary production is threatened by the changed climate (Lobell et al., 2011).

Securing the Future Primary Production

Considerable ongoing work focuses on the many aspects of securing the future primary production. The demand to primary production is much influenced by consumer habits and trends as well as economical interests and governmental legislation. Further, distribution of and economic access to the primary production complicate its security. In addition, political decisions are to reach much farther into the future than the norm and approaches to limit emissions of greenhouse gasses should be global due to the common atmosphere and world markets (Gregory et al., 2005; Baethgen, 2010). Hence, securing the primary production is complicated, however, considering the projected decrease in yield and the lowered predicted increase rate (Fig 2), it is essential to take action.

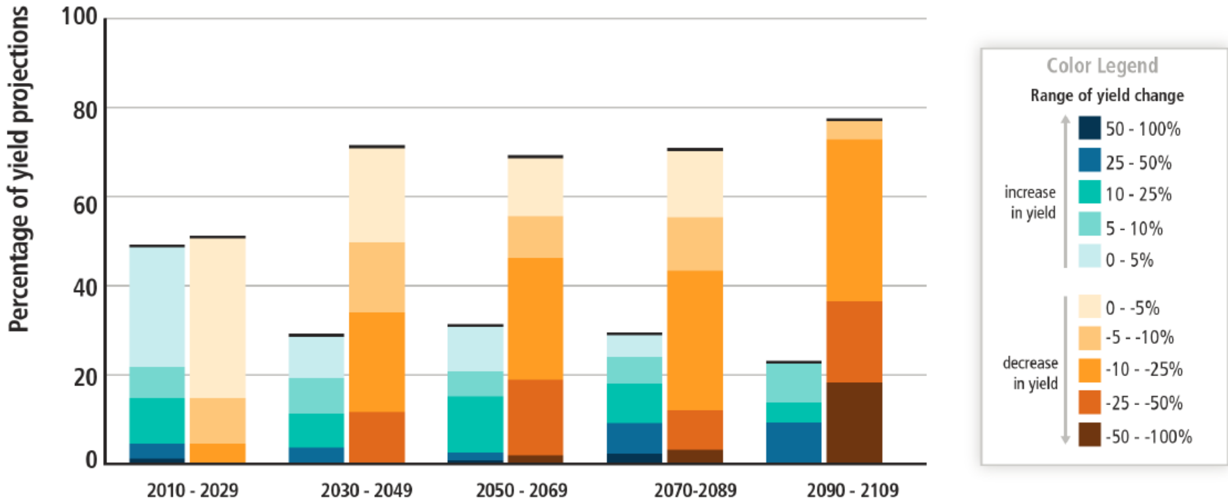

Figure 2. Projected changes in crop yield caused by climate change summarized from tropical and temperate regions and for adaptation and non-adaptation cases. From IPCC AR5 WG II summary, Fig SPM.7 (2014b).

Mitigation of the projected decreased primary production is approached with different adaptation strategies. At the farms adaptation can be attempted by changes of the existing cropping system e.g. sowing date, changing crop species or by cultivate dry-land (Rickards and Howden, 2012; Challinor et al., 2014). A second level of mitigation of climate change effects on primary production is genetic by adaptive evolution with spontaneous genetic changes (Ackerly et al., 2000; Matesanz et al., 2010). Finally, an approach could be to decrease the demand on primary production by either lowering meat consumption or by in vitro meat production (Post, 2012).

Adaptation of cropping systems

Adaptations in the cropping system have been reported promising in mitigation of negative effect by climate change on the primary production (Olesen et al., 2011; Anwar et al., 2012). Experimental studies have demonstrated time of sowing, practices in soil cultivation and irrigation to possess potentials in mitigating climate change effects on cereal grain yield (Tewolde et al., 2006; Branca et al., 2013; Lehmann et al., 2013). In the temperate regions, the reported 15 % decreased grain yield of wheat was suggested to be turned into a 10 % increase through adaptations to the cropping system under a +5 °C scenario (Challinor et al., 2014). That the effect of adaptation is complicated to asses is emphasised by a discrepancy of 15 % from the previously reported decreased grain yield of wheat under +5 °C in 2007 (IPCC, 2007) to the increase now reported (Challinor et al., 2014). The projections are from similar methods, but the latest finding of 10 % increase also includes additional experiments performed in the time interval 2007-2014. Despite that adaptations in the cropping system are promising, IPCC (2014b) states that there are limits to the effectiveness of such adaptation as also emphasized in Figure 2.

A considerable limitation in the assessed effects of adaptation is that extreme events have not been a factor in the projections (Challinor et al., 2014). The absence of such a major factor of future climate change calls for studies that experimentally assess the effects of extreme climate events on crop yield.

Introduction of elite cultivars to the northern region from the southern could be an approach to mitigate lowered grain yield from increasing temperature, however, grain development is dependent of flowering. Time of flowering is complexly regulated from numerous pathways with one being photoperiod, that differs widely from south to north. Hence, adaptation of photoperiod is necessary, when introducing southern elite cultivars to the long days of the north (Milec et al., 2014). Craufurd et al. (1999) identified the adaptation of Sorghum to its broad geographical regions to be determined by photoperiod sensitivity, suggesting an adaptation potential.

Adaptation of cultivars

Adaptive evolution studies suggest the pace of climate change to outperform the spontaneous genetic changes (Rosenzweig and Parry, 1994; Potvin and Tousignant, 1996; Alemayehu et al., 2013; Svenning and Sandel, 2013) with consequently lowered mitigation of climate change effects on primary production. However, performing the perfect selection experiment under field conditions is impossible due to the nature of the unknowns of climate change e.g. pace of the increase in climatic factors from one growing season to the next, occurrence, length, levels and timing of extreme events as well as climate effects on pest, pathogens and soil properties. The great climate change field experiment that we are all a part of is naturally progressing, but leaving us little chance to take advantage of knowing what to come. Selection experiments in enclosure studies have the risk of including adaptation to the enclosure environment, but are, however, for the time being the best possible way.

In a unique study by Nevo et al. (2012) the effect of 28 years of global warming was reported in wild relatives of wheat and barley by comparing material sampled 28 years ago with present material. The wild relatives were found to shorten time to flowering by 10 days indicating that adaptation of photoperiod response is ongoing; also depletion of allele number was observed. The study by Nevo et al. (2012) were performed in wild material that potentially have a wider genetic base than crops, and therefore perhaps a larger potential for genetic adaptation compared to cultivars, which might have experienced the much discussed genetic bottleneck during domestication (Tanksley and McCouch, 1997; Malysheva-Otto et al., 2007). Adaptive evolution relies on a genetic base from which it can develop, and sexual reproduction to expand that genetic base. In plant breeding both the genetic base and sexual reproduction are managed in the development of new cultivars. Plant breeding can also benefit from genetic resources that are stored in genebanks (FAO, 2013). These might help broadening the genetic base and possess valuable sources for climate resilience.

Breeding of Future Cultivars

Irrespective of the pace of climate change and mitigation by adaptation, the abilities of the cultivars used will be determining for primary production. This has also recently been emphasized in modelling studies (Challinor et al., 2014; Martín et al., 2014). Within the Nordic regions agricultural production must be maintained and preferably improved to compensate for decreases in other regions. The goal is high yielding cultivars under the future biotic and abiotic stress in low input regimes.

Significant progress in breeding started in the 1960s, and has become known as the 'Green Revolution' generating substantial improvement of cultivars. The improvements were among others caused by introduction of dwarfing genes as well as exotic plant material introducing disease resistance, and by accelerating the time of cultivar development and broadened adaptation by introduction of shuttle breeding (Evenson and Gollin, 2003; Hedden, 2003). The current situation with climate change and growing world population calls for a new 'Green Revolution' introducing cultivars resilient to abiotic stress in addition to the biotic, which is also impacted by climate changes. The projected demand on the primary production in 2050 is reported to be an additional 46 % to 70 % or even larger (FAO, 2009; Tester and Langridge, 2010; Tilman et al., 2011; Lobell, 2013). A safe statement is to say that the growth rate of primary production must substantially increase compared to now. An added challenge with respect to the primary production is the demand for sustainability, which was not included in the first 'Green Revolution' (Evenson and Gollin, 2003). However, developments in genomic technologies such as sequencing (Davey et al.,

2011) and high-throughput array based marker genotyping have been rapid (Close et al., 2009). In addition, the development has lowered the cost for genotyping (Wetterstrand, 2014). Together with the line of –omics the new technologies can all detriment the insight into genomic variation and exploitation of the available plant genetic resources when phenotype is known (Varshney and Dubey, 2009). Further, political focus on the needed action to secure future primary production seems to grow (Gaffney, 2014).

The improvement of resilience to abiotic stress is challenged by the efforts demanded for the phenotyping. Abiotic stress affects crop performance differently, depending on its timing, frequency and intensity. Further, transfer of undesirable genes linked to the preferred trait for abiotic stress impedes the breeding progress (Varshney et al., 2011). Marker assisted selection developed from the numerous DNA marker types emerging in the1990's, and their incorporation into PCR-based methods (Staub et al., 1996) are with the lowered cost widely used. Statistical methods as association mapping (Gupta et al., 2005) and genomic selection (Heffner et al., 2009) have developed to link the polymorphisms found by the genotypic data systems with available phenotype data. Both methods take advantage of linkage disequilibrium between alleles and can be applied in diverse sets of accessions. Association mapping identifies quantitative trait loci and genomic selection predicts breeding values of lines from the included phenotypes and genome-wide markers applied (Gupta et al., 2005; Heffner et al., 2009). Genomic selection has with success been applied in animal breeding (Hayes et al., 2009). If it will be common also in barley breeding will be interesting to follow. One challenge is the fundamental differences of the phenotyping recorded in animal breeding and in plant breeding; in animal breeding, few male breeding animals are use and the offspring thereof continuously phenotyped (Jonas and de Koning, 2013). Association studies are, however, widely applied in barley (Kraakman et al., 2004; Tondelli et al., 2013; Visioni et al., 2013).

All together, the breeding goals of the future are not that different from the breeding goals of today; high yield. The challenge though lies in the lack of easy screening methods to select the best performing cultivars under the future climate, as in the knowledge gap on the plant responses to combined climatic factors.

Barley

The cultivated barley (*Hordeum vulgare*) is breed from wild barley (*Hordeum spontaneum*), which can still be found in the Fertile Crescent in the Middle East. Throughout cultivation

tetraploids (*2n = 4x = 28*) have appeared and are still present within the genus, but the barley cultivated today is diploid (*2n = 2x = 14*) and self-pollinated (Harlan, 1976). Crosses can be performed freely between the wild barley, landraces, varieties and barley cultivars of today (Backes et al., 2006) securing easy introduction of genetic diversity and use of plant genetic resources. Natural diversity of barley is well represented within genebanks that holds approximately 400,000 accessions worldwide. Hereof 290,000 are classified into wild relatives, landraces, breeding/ genetic stocks and cultivars, and most often further characterisation is absent (Global strategy for the ex situ conservation and use of barley germplasm, 2008). Barley is grown worldwide in high and low input agriculture, at the lowest seacoast and on the highest mountain, from the northernmost arable land to the tropics, as elite cultivar or as landraces demonstrating adaptability to several environments (Newton et al., 2011). Worldwide, barley holds fourth place in terms of area cultivated and production after wheat, rice and maize. Despite the early use of barley in human diet (Kislev et al., 1992) todays use is mainly as feed for livestock and malt for use in the brewing and distilling industries – the two different applications hold different requirements to quality. Today human diet constitutes just 0.2 % protein (g/capita/day) from barley worldwide. In comparison wheat, maize and rice contributed with 16 %, 10 % and 3.4 % of the protein respectively in 2009 (FAOSTAT, 2014). However, in recent years barley has received renewed interest as food due to its possible health benefits and nutritional value (Baik and Ullrich, 2008).
On European fields barley production is among the highest yielding; 6 tonnes per hectare compared to 2 tonnes per hectare from the major producers Russia and Australia, where the production system is though also less intensive (Newton et al., 2011). Europe was among the world's main importers of barley in 2009, however, France, Germany, Ukraine, Spain and Denmark was also among the 10 main exporters, maybe to Saudi Arabia, which was the lead barley importing country (Newton et al., 2011). In the Nordic countries Denmark, Finland, Norway and Sweden, barley was the main grown crop in 2012 and constituted $1,311 mio of the agricultural GDP in Denmark (Danmarks statistik, 2014). However, yields are stagnating for barley as well as wheat (Fig 3) and actions are to be taken.

Barley vs wheat

One could speculate if barley in the future could advance from a fourth place in 'major cereals of the world' due to a conceivable greater potential for improvement in terms of low input production and nutritional profile for human consumption. Today, the three major cereals in world production are maize, rice and wheat (FAOSTAT, 2014), and wheat (most similar to barley) ranking third (Shiferaw et al., 2013).

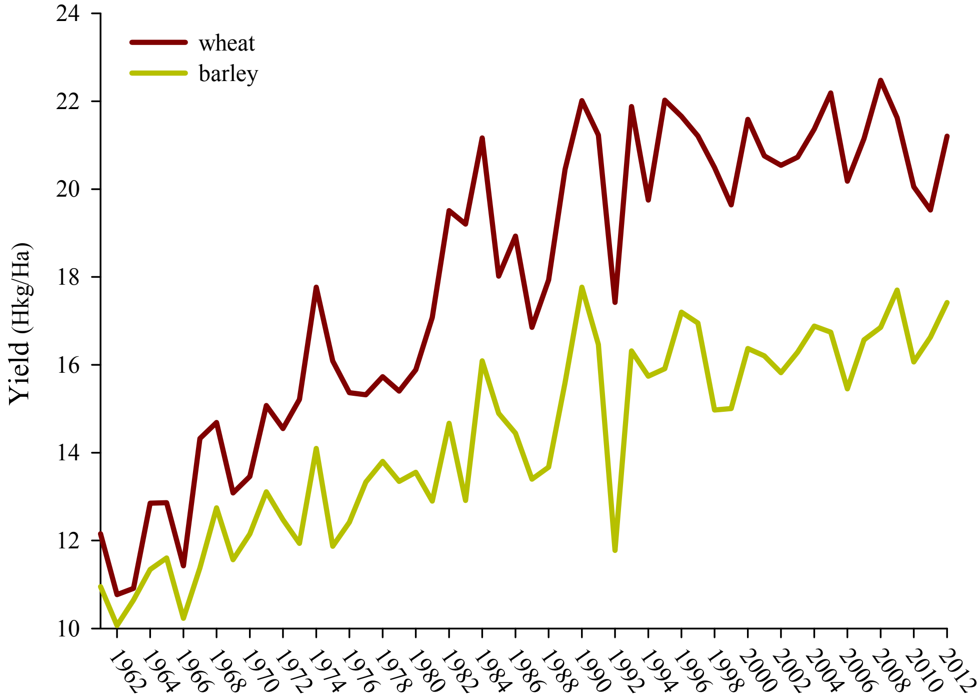

Figure 3. Barley and wheat yield in Denmark, Norway, Finland and Sweden from 1961 to 2012. Generated from FAOSTAT data (2014).

In terms of low input production, barley was during the 'Green Revolution' targeted much less than wheat, maize and rice, where increased yields were achieved with little focus on sustainable production (Evenson and Gollin, 2003), however, barley follow the increase in wheat yield in Denmark, Sweden, Norway and Finland (Fig 3). Further, barley is frequently cultivated in low input systems also of no irrigation, why genetic resources are available. With regard to nutritional value, barley holds great potential due to balanced protein composition with essential amino acids and high levels of minerals, antioxidants and levels of β-glucans (Baik and Ullrich, 2008). The baking quality of barley has room for improvement compared to the one of wheat, however, up to 15-20 % barley flour blended into wheat flour was found to have no influence on quality (Dhingra and Jood, 2004). In addition, baking quality has not been targeted in barley breeding. Apart from barley having potential in relation to sustainability and nutrition, barley has the reputation to possess high stress tolerance, possible from its wide geographical distribution, which could be the trumph to reach first place as major cereal in the future changed climate. The reputation of barley holding high stress tolerance was though challenged by Cossani et al. (2009) under Mediterranean growing conditions with various levels of water and nitrogen regimes. Barley was not found to have a clear yield advantage over wheat in these stressful environments. However, as noted by Newton et al. (2011) the wide geographical distribution of barley has led to local adaptation, consequently with local solutions and additional increase of resilience.

The frame of the present PhD project and some retrospective reflections

The work-frame of the present PhD project has been the Technical University of Denmark, Campus Risø within the Centre for Ecosystems and Environmental Sustainability (ECO) and the network 'Sustainable primary production in a changing climate'. This frame secured access to numerous experts on plant science, and infrastructure facilities capable of mimicking climate change conditions in plant experiments.

The 'Sustainable primary production in a changing climate' network

The 'Sustainable primary production in a changing climate' network (http://www.sustain-nordforsk.kt.dtu.dk/) was established in 2011 for 3½ consecutive years, and comprised plant scientists from universities and plant breeders from companies in Denmark, Norway, Finland and Sweden as well as scientists from NordGen (the Nordic Genebank) with a total of 10 participating institutions/companies. Among other aims, the network tried to prepare for tomorrow's changed climate, by searching for resilient barley varieties to be introduced in the work of the Nordic breeders to mitigate climate change effects on primary production. Further, the results generated within the network have been used in life cycle assessment analysis (LCA) of the environmental consequences of the future barley production. Results have also delivered recommendations to Nordic politicians within the agricultural sector.

Within the network, dynamic energy has followed a true collaboration from the generation of ideas to practical tasks as phenotyping. Linking industrial partners to academic research has in this network been fruitful for all partners emphasized by its productivity; 3 published papers plus 9 manuscripts. Also new collaborations e.g. on breeding for insect tolerance in a changed climate have emerged from the network. As the PhD of the network I have received inspiration, experienced different viewpoints and had numerous experts to consult in many aspects of plant science. I have also, on close hand, witnessed a growing interest from the breeders in genetic resources for climate resilient cultivars.

RERAF - a climate phytotron

RERAF (Risø Environmental Risk Assessment Facility) is a unique research facility, where atmospheres and other environmental components can be combined, controlled and continuously monitored (http://www.eco.kt.dtu.dk/Research/Research_Facilitites/RERAF). Six individually controlled 24 m^2 gastight chambers can be supplied with up to 10 different gasses. Together with control of humidity, temperature and light, the desired atmospheres can be mixed. The size of the chambers enables population studies, and other experiments with large quantities of plants.

In the experimental work of the present dissertation temperature, [CO_2] and [O_3] were elevated as single-factor treatments, in a two-factor treatment of temperature and [CO_2] in combination and in one experiment an extreme heatwave was applied in addition to the climate treatments ([O_3] excluded). As control treatment (amb) ambient present south Scandinavian summer was mimicked. The set points of the experiments were according to Table 2. The climatic factors were elevated constantly with a day/night regime for temperature as for humidity and light (18/6 h). Humidity and light were also set at constant levels. Water was applied at the same amount over all treatments early in the daytime regime at a level recognised for optimal development under ambient conditions. In the treatments of elevated temperature, the adult plants most likely experienced limited water availability due to increased vapour pressure deficit and following evaporation. Throughout the lifecycle of the plants, the growing conditions were strictly controlled at levels stated in Table 2.

The highly controlled environment of the climate phytotron is a great advantage, however, at the same time a disadvantage. In the search for crop genetic resources the obvious final use is under field conditions, with no walls preventing e.g. wind, clouds, pest and pathogens. Further, the pot environment with its root constraints and artificial soil temperature is different from field conditions. FACE (free air carbon enrichment facilities) have proven successful in experiments with elevated [CO_2] and similar free air fumigation systems for [O_3] and their combination (Fuhrer, 2003; Ainsworth and Long, 2005). Considering climate change, the global warming is considered a main driver, why elevated temperature should be included in climate change experiments, and only temperature elevation of 1-3 °C seems possible under in FACE experiments under field conditions (Kimball et al., 2007; Bruhn et al., 2013). Hence, in terms of temperature increase above a couple of degrees Celsius, highly controlled enclosure studies have their advantage. Another benefit of enclosure studies is repeatability, which is higher than under field conditions. A future experiment to perform would be to stagger the field conditions within RERAF including diurnal rhythm and cloud cover by light intensity changes (to be installed).

Table 2. Set point values for temperature (tmp), [CO_2] (CO_2), [O_3] (O_3) and humidity in experiments of the present dissertation, Ambient: amb, elevated [CO_2]:+CO_2, elevated temperature: +tmp, elevated [O_3]: +O_3. heatwave: +H.

	Tmp day/night	CO_2 (constant)	O_3 (constant)	Humidity (day/night)
amb	19/12°C	385/400 ppm	none added	55/70
+CO_2	19/12°C	700 ppm	none added	55/70
+tmp	24/17°C	385/400 ppm	none added	55/70
+tmp & CO_2	24/17°C	700 ppm	none added	55/70
+O_3	19/12°C	385/400 ppm	100-150 ppb	55/70
+H	33/28°C	385/400 ppm	none added	55/70
+H & CO_2	33/28°C	700 ppm	none added	55/70

Despite the artificial conditions in the pot setup, plant competition was included throughout the experimental work of the present dissertation by growing eight individuals together in 11 L pots with a plant density of 151 plants/m^2. This is a plant density lower than recommended in the field, but grain yield in barley seems rather unaffected by lowered plant density of this range (Schillinger, 2005). Regardless of the lower plant density final biomass exceeded the recommendation of 1 g L^{-1} pot (Poorter et al., 2012). As expected the yield achieved within RERAF was considerable larger than what was achieved from the same accessions cultivated under field conditions.

The NordForsk-accessions

A diverse set of 140 spring barley accessions was supplied from partners of the network with the majority from NordGen. The set comprised 15 2-rowed and 33 6-rowed landraces, 61 2-rowed and 24 6-rowed cultivars, four 2-rowed and one 6-rowed breeder-line and two accessions that showed to be winter barley and were excluded. The cultivars spanned the period 1883-2013 in year of marked release with the majority (53) from after 1975. Geographically, most accessions were of Nordic origin, some were European and six landraces were exotic e.g. from Nepal and Afghanistan. The apparent morphological diversity observed in the experiments was remarkable (for full list of accessions, please see S1 of manuscript 1).

Considering the geographical origin of the included accessions, the ambient treatment mimicking southern Scandinavian summer has not been optimal for all accessions included. A common denominator is challenging to asses even within the Nordic region, due to the great differences in light regime and duration of the growing season.

The network accessions were picked by NordGen to be representative for the barley material in the genebank. With the knowledge gained throughout the last three years and in the light of the abilities of GWAS, I would, if I could do it all again, either increase the set of accessions or limit the genetic structure. Both approaches could potentially increase the frequency of alleles to a level detectable by the statistical tools.

Research approach of the present PhD project

In the present PhD project, two main experiments (Table 3) investigated the effect of projected climate change on numerous genotypes with the aim of contributing to mitigation of decreased future primary production by 1) mining genetic resources that potentially, can be exploited in breeding 2) add basic knowledge on plant response to combined and extreme climate treatments and 3) supply input data on the variable primary production and quality for use in modelling studies to predict future climate effects to cropping systems. The experimental data are reported and discussed in four manuscripts (Tabel 3). In conclusion, climate change effects were found to decrease grain yield substantially under climate change conditions, however, vast variation was identified in the studied accessions.

Table 3. Main experiments and manuscripts of the present PhD project.

Main Experiments
• 140 spring barley accessions grown under future climate change conditions
• 22 spring barley accessions exposed to heatwave in addition to future climate change conditions
Manuscripts
1) Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions
2) Genome-wide association study of production and stability traits in spring barley cultivated under future climate scenarios
3) Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 barley (Hordeum vulgare L.) accessions
4) Effect of an extreme heatwave on 22 spring barley accessions cultivated in future climates. Tendencies in allocation of biomass, temperature priming, CO2-responsiveness and stability of grain yield

Additional screen of NordForsk-accessions for disease resistance, nutritional value and expanded use of generated data

In collaboration with the breeders of the network the NordForsk-accessions were screened for resistance to the following fungal diseases: leaf rust (*Puccinia hordei*), net blotch (*Pyrenophora* *teres* f. *teres*), ramularia (*Ramularia collo-cygni*), scald (*Rhyncosporium secalis*) and spot blotch (*Cochliobolus sativus*). The screening for resistance was performed in the field or in enclosure facilities of the breeding stations. Ramularia, scald and net blotch are considered emerging pathogens in the Nordic region (pers. comment L. Reitan, M. Jalli and J. D. Jensen, GramiNor, MTT and Nordic Seed respectively).

The screen for resistance to net blotch was performed under field conditions in Finland (GPS: 67.47476N, 30.8587E) by me at my external stay. Individual plants, ten of each cultivar and 50 of each landrace, were scored four consecutive times. The scoring was performed at the adult stage three times, recording the percentage of leaf area damaged (1-100 %) on the 2nd and 3rd leaves and the area under the disease progress curve was calculated. A modified Tekauz scale 1-4; 1 fully resistant (Tekauz 1-2), 2 medium resistant (Tekauz 3-5), 3 medium susceptible (Tekauz 6-8), 4 susceptible (Tekauz 9-10; Tekauz, 1985) was used in the fourth scoring of individual accessions. Variation was identified in resistance of the included accessions, however, none of the accessions were fully resistant under the high disease pressure of the season in question (pers. comment M. Jalli).

A genome-wide association study (GWAS) has been performed with the phenotypes scored in all disease screens and single nucleotide polymorphism (SNP) markers (Illumina array). In this GWAS, novel marker-trait associations for spot blotch and ramularia were identified (Table 3) supplying markers for use in the breeding for resistance. Manuscript preparation in collaboration with G Backes, M Jalli, L Reitan, MF Lyngkjær, P Peltonen-Sainio and RB Jørgensen is in progress.

Table 3. Marker-trait associations identified for the fungal diseases leaf rust, net blotch, ramularia, scald and spot blotch. Chrom: chromosome with bin position and Pos: position

SNP marker	Disease	Chrom	Pos (cM)
i_SCRI_RS_14227	Ramularia	1H.3	27.50
i_SCRI_RS_155382	Ramularia	1H.3	28.40
i_SCRI_RS_221814	Leaf Rust	3H.2	17.40
i_SCRI_RS_151868	Net Blotch, time 2	3H.10	139.30
i_12_30718	Spot Blotch	4H.6	97.20
i_12_30635	Net Blotch, time 2	5H.8	128.20
i_SCRI_RS_222984	Net Blotch, time 2	5H.8	128.20
i_SCRI_RS_228463	Net Blotch, time 2	5H.8	128.20
i_11_20746	Net Blotch, time 2	6H.6	72.90
i_SCRI_RS_42792	Scald	7H.1	4.10
i_SCRI_RS_29353	Spot Blotch	7H.2	23.80

Grains of a subset of 22 accessions, selected from their performance in the disease screen and response to the climate treatments in RERAF, were further screened for contents of phytic acid (PA-P), zinc and iron content under all applied climate treatments. The purpose was to explore climate change effects on nutritional values. In grains, PA-P is formed during ripening and presents 60-90 % of the phosphate (Loewus, 2002). PA-P is found to have opposing nutritional effects making it widely studied (e.g. Harland et al., 1995; Kumar et al., 2010). Firstly, PA-P is suggested to have anticancer and antioxidant effect on monogastric species as well as positive effect on coronary diseases and diabetes. Secondly, PA-P is found to inhibit the uptake of minerals as zink and iron in monogastric species potentially leading to malnutrition (Greiner et al., 2006). Manuscript preparation in collaboration with RB Jørgensen, AM Torp and SK Rasmussen is in progress.

Throughout the time of the experimental work of the present dissertation a FACE facility was established at DTU, Risø, Roskilde. A subset of ten cultivars, selected from their performance in the disease screens and response to the climate treatments in RERAF, were cultivated in four octagons of ambient (396 ppm) and four octagons of above ambient (510 ppm) [CO_2] in the growing season 2013. Grain yield, above ground vegetative biomass, HI, TGW, and plant height were analyzed together with presence of fungal diseases. In addition water use efficiency was assessed in six cultivars by measurements of the $^{13}C/^{12}C$ isotope ratio in leaf material. Manuscript preparation in collaboration with H. Bøg, P. Ambus, TN Mikkelsen, MF Lyngkjær, G. Backes and RB Jørgensen is in progress.

The grain yield data of a representative subset of cultivars analysed in the climate treatments of RERAF (13 accessions) together with grain protein concentrations of this subset were included as "primary data" in a life cycle assessment (LCA) study. The LCA study assessed the eco-efficiency "from a cradle to farm gate" perspective of spring barley production under climate change conditions. The experimental data provided a rare opportunity to perform LCA with measured (and not modelled) input data describing future conditions. Manuscript in collaboration with M Niero, M Z Hauschild, MF Lyngkjær, P Peltonen-Sainio and RB Jørgensen has been submitted to Global Change Biology.

Strength and limitations of experiments in the present PhD project

In all experiments of the present dissertation plants have been grown from seeds to maturity and the produced grain yield assessed, and I consider this "life cycle approach" a great strength. Very often effects are only studied during part of the life cycle. The experiment of the present dissertation also deviate from other studies of climate change effect to plants with respect to the number of genotypes studied; Grain yield has been assessed in a rather large set of 22, 108 or 138 individual accessions. As mentioned the choice of cultivars has recently been reported a cornerstone in securing the future primary production (Challinor et al., 2014; Martín et al., 2014). The results of this dissertation support the large genetic potential for securing the future primary production. The often reported increase in grain yield from elevated [CO_2] (Amthor, 2001) and decrease by elevated [O_3] (Fuhrer, 2009) were not existing for all accessions, however, for barley grain yield overall, these trends were observed. Likewise, elevated [CO_2] was not found to increase the grain yield of all accessions. These differences, with their underlying genetic diversity, are the starting point for breeding of climate resilient cultivars. However, we can only use that diversity if we know of it. With the entrance of high-throughput and low cost genomic methods the limiting factor is becoming the phenotyping, as also emphasised by Cobb et al. (2013). Studies reporting physiology measures at vegetative stages on a single genotype, does not contribute to extracting the available diversity to be exploited in securing the future primary production.

In 1991 Lawlor and Mitchell emphasised the need of studies assessing the effect on grain yield by the combination of elevated temperature and [CO_2]. In the recent assessment report of IPCC the projections of the future climate effects rely on modelling studies based predominantly on single-factor treatments. I consider it a strength that the accessions of the present experiments all have been screened for their response to the combined elevation of temperature and [CO_2]. Further, the stability over treatments has been accessed with the stability indices environmental stability (S^2) and Wricke's ecovalence (W^2) (Annicchiarico, 2002). The stability is though calculated over climate scenarios that most likely will not occur, e.g. that only [CO_2] increased is very unlikely. Establishing the stability of accessions cultivated in different multifactor treatments would be highly interesting. From this it follows that I find it arguable if screens using single-factor treatments are relevant for revealing genetic resources to be exploited in breeding of future cultivars.

It is a limitation of the work of the present PhD that no molecular, biochemical or physiological measures systemically have been performed during the experiments. Simple measurements as development scores, photosynthetic parameters or leaf samples for later processing, could have added significant value to the production parameters assessed. However, it might be symptomatic; experiments may be either focused on physiology including one or few genotypes, but applying numerous methods or as in the present dissertation numerous genotypes is another route to go. More repetition of performed experiments would also have increased the scientific credibility. Possible chamber effects have been diminished by rotation of treatments with corresponding batches of plants between chambers. Nevertheless, throughout plant development sensitivity to external stimuli varies and the different treatments with corresponding plants have not been placed in the same chamber at the same time of development.

The single-factor treatments of elevated [O_3] has throughout the process of the present dissertation somehow slipped into the background. A plausible cause is its absence in the combined treatment and not because [O_3] is less relevant. The optimal, traditional experimental setup with all combinations of a triple-factor treatment would have taken up more chambers than available; (1) ambient, (2) temperature, (3) [CO_2], (4) [O_3], (5) temperature and [CO_2], (6) temperature and [O_3], (7) [CO_2] and [O_3] and (8) temperature, [CO_2] and [O_3]. Further I decided on a reserve chamber in case of a chamber breakdown. Within RERAF, O_3 is applied by generators in the chambers and input manually adjusted and due to the high reactivity of O_3 its concentration is more challenging to adjust than CO_2. Hence, to decrease the work load [O_3] was only applied in one treatment. However, the effect of elevated [O_3] in combination with climatic factors on production parameters is highly relevant and should be included in assessments of projections for future primary production as also emphasized by Ainsworth et al. (2012). That [O_3] influence growth was found in one of the excluded winter barley accessions, which only under elevated [O_3] produced ears on all eight plants.

Novel findings

1) A general decrease of 29 % in overall grain yield of 138 spring barley accessions cultivated in a future changed climate scenario of elevated temperature and [CO_2].

2) A general decrease of 23 % in grain protein harvested of 108 spring barley accessions cultivated in a future changed climate scenario of elevated temperature and [CO_2].

3) A general decrease of 52 % in overall grain yield of 22 spring barley accessions cultivated in a future changed climate scenario of elevated temperature and [CO_2] with an induced heatwave of 10 days around flowering.

4) Vast variation in response to all applied treatments in the set of 138 spring barley accessions.
Accessions that maintained grain yield under the combination of elevated temperature and [CO_2] were identified.
Accessions with increased grain protein harvested under the combination of elevated temperature and [CO_2] were identified.

5) Candidate markers for exploitation in breeding of the variation identified under climate change conditions, e.g. response to elevated [CO_2] and climate stability of grain yield.

6) Strong indications that primary production under future climate conditions cannot be projected from the single-factor treatments. Supported e.g. from the findings of grain yield under the combined treatment being different from the added decrease/increase under the elevated temperature and [CO_2], and supported from marker-trait associations in the double factor treatment being different from those found under single-factor treatments.

7) Elevated temperature prior to an extreme heatwave decreased the strong effect of the heatwave, whereas prior elevated [CO_2] increased the effect of the heatwave on grain yield.
The effect of a heatwave to grain yield was found to be similar under ambient conditions and under a future scenario of elevated temperature and [CO_2].

8) Tendency of accessions with high response in grain yield under elevated [CO_2] as single-factor to produce high grain yields under elevated temperature and [CO_2] with additional heatwave.

Outlook

To what extent climate change progress and agriculture adapt will have consequences for the future primary production. To assess the possible consequences, climate change effect on crops should be estimated from treatments as realistic as possible. It is acknowledged that climate change includes increase in multiple climatic factors, and that within-season variability most likely will increase. Hence, climate change experiments should be multi-factorial and include extreme events; besides large sets of accessions of individual crop species should be targeted in the experiments. This could further improve model studies estimating the effects of climate change on the primary production. With the numerous high-throughput systems for genotyping and decreasing costs, the phenotyping is becoming the limiting factor. However, phenotyping of numerous genotypes in terms of quantity and quality characteristics should be performed and associated with easy-to-use DNA markers.

Without doubt, the most realistic future scenario could be mimicked under field conditions, why continuous development in establishing techniques to increase temperature in FACE experimental setups is highly encouraged. Also development of efficient infrastructures of experimental facilities could encourage exchange of knowledge and lead to results faster.

Variety mixtures are with regard to market demands not favourable being too heterogeneous in quality. However, they might be beneficial in securing a stable production in a future variable climate as they often represent a broader genetic base than a single cultivar, and thus might have a buffer capacity in the case of extreme environmental challenges.

A plant organ rarely considered in climate change experiments is the hidden root. Undoubtedly roots are also impacted by changes in climate, shifting soil characteristic and they determine the plant performance. The roots are potentially a subject for improvements.

Was it not for our dependence on the world and its resources, climate change would have been a fascinating phenomenon to follow. The primary production is a resource that humankind is highly dependent on, why continuous and relevant research should continue.

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MANUSCRIPTS

1. Ingvordsen CH, Backes G, Lyngkjær MF, Peltonen-Sainio P, Jensen JD, Jalli M, Jahoor A, Rasmussen M, Mikkelsen, TN, Jørgensen RB. **Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions.**

   The manuscript has been submitted to European Journal of Agronomy March 2014.

2. Ingvordsen CH, Backes G, Lyngkjær MF, Peltonen-Sainio P, Jahoor J, Mikkelsen TN, Jørgensen RB. **Genome-wide association study of production and stability traits in spring barley cultivated under future climate scenarios.**

   The manuscript is intended submitted to Molecular Breeding.

3. Ingvordsen CH, Gislum R, Jørgensen JR, Mikkelsen TN, Jørgensen RB. **Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 barley (*Hordeum vulgare* L.) accessions.**

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4. Ingvordsen CH, Lyngkjær MF, Peltonen-Sainio P, Mikkelsen TN, Jørgensen. **Effect of an extreme heatwave on 22 spring barley accessions cultivated in future climates. Tendencies in allocation of biomass, temperature priming, CO_2-responsiveness and stability of grain yield.**

   The manuscript is intended submitted to Journal of Experimental Botany/Global Climate Change.

Manuscript 1

Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions

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Highlights

- 138 barley accessions screened under future levels of [CO_2], [O_3] and temperature
- The effects of the single climatic factors were not found to be additive
- Climate-stable and high yielding accessions were identified

A B S T R A C T

The present study identified spring barley genotypes possessing genes that can be exploited for breeding of stable and high yielding cultivars under future climate conditions. A comprehensive set of 138 spring barley accessions, landraces, cultivars and breeder-lines, were during their entire life cycle cultivated in large chambers of a climate phytotron. The most realistic climate scenario applied was a two-factor treatment of elevated temperature (24 °C/17 °C) and atmospheric carbon dioxide (700 ppm) and the control treatment was equivalent to present average South Scandinavian climate (temperature: 19 °C/12 °C, [CO_2]: 385 ppm). All 138 accessions were also exposed to single factor treatments of elevated temperature (24 °C/17 °C), [CO_2] (700 ppb), and [O_3] (100-150 ppb). A decrease of 29 % in overall grain yield was found in the two-factor treatment. However, accessions that maintained grain yield in the two-factor treatment and demonstrated yield stability over treatments were identified both among old cultivars (1883-1974), modern cultivars (1978-2013) and breeder-lines. Further, accessions with unchanged or with increased grain yield were identified from the single factor treatments. Aboveground vegetative biomass was overall not affected in the two-factor treatment. From our findings we recommend that stability indexes are combined with yield measurements in the search for germplasm to secure a stable future production in a changed climate. Also, on the basis of the present experimental work we argue that analysis of genetic resources for future environments should be performed under multifactor climate conditions, as single factor treatments rarely allow qualified forecasting.

1. Introduction

Climate change alters growth environments around the world and challenges the agricultural production. At the same time the world population is growing with the need of an increased food production as consequence. Unprecedented climates are reported to occur around 2047 (+/- 14 years; Mora et al., 2013) and already by now actual levels of elevated temperature and increased atmospheric concentrations of carbon dioxide ([CO_2]; 400 ppm) and ozone ([O_3]; 32-62 ppb; Ellermann et al., 2013) have impacted cereal yields (Lobell & Field, 2007; Lobell et al., 2011; Trnka et al., 2012). By the end of the 21st century temperature is expected to increase by 3-5°C according to a worst-case scenario (RCP8.5), [CO_2] to reach 1415-1910 ppm and [O_3] to increase by 25 % compared to the concentration experienced today (IPCC, 2013). A recent study suggests that the worst-case scenario is very probable the one to expect (Sherwood et al., 2014). Climate change is further projected to increase the frequency of extreme events such as floods, heat waves, droughts and storms with great risk to further decrease the crop yield (IPCC, 2013; UNFCCC, 2010). Under mid to high latitude conditions temperature increases exceeding 2°C is expected to reduce cereal yields (IPCC, 2007). The Nordic agriculture is further in risk of summer drought and heavy rains due to changed precipitation patterns (Christensen et al., 2011; Högy et al., 2013). The rapid changes in growth conditions induced by altered climatic conditions urge the need to develop climate resilient cultivars through plant breeding and apply new management practices (Anwar et al., 2013).

Worldwide, barley is the fourth most important crop and has over the last 40 years - together with the other major cereals - experienced around 50 % increase in production as a result of greater input of fertilizer, irrigation, pesticide application and the introduction of new cultivars (Tilman et al., 2002). The annual growth rate of the global agricultural production was 2.1 % on average from 2003-2012. However, the annual growth rate is expected to decrease to 1.5 % per year in the coming decades (OECD/FAO, 2013). The future lower growth rate is due to limited expansion of agricultural land, rising production costs, restricted use of non-renewable resources together with reduced use of fertilizer and pest control agents to limit their environmental side effects (Foley et al., 2011; OECD/FAO, 2013; Tilman et al., 2001). Plant breeding has the enormous task to increase the future primary production. In this context, gene bank material and exotic germplasm can possess specific genes and genetic diversity, which can be exploited in the development of stable and high yielding climate resilient cultivars. However, the limited information available on climate tolerance within plant accessions, is an obstacle for their direct use (Ceccarelli et al., 2010; Newton et al., 2011), not to mention the complexity in the search for tolerance to climates not earlier experienced by any crop. As emphasized by Powell et al. (2012), the utilization of genetic resources with tolerance to climatic factors is impeded by lack of reliable and cost efficient screening methods.

It is found that various crop species respond differently to the factors of climate change (e.g. Feng and Kobayashi, 2009; Kimbal, 1986; Luo, 2011; Mills et al., 2007). However, the level of variation within the genotypes of a crops has been less studied (e.g. Craufurd et al., 2003; Pleijel, 2011; Weigel and Manderscheid, 2012), and rather few studies have investigated the effects of treatments, where more than one climatic factor was manipulated at a time (Alemayehu et al., 2013; Clausen et al., 2011; Frenck et al., 2011; Juknys et al., 2011; Kasurinen et al., 2012; Mitchell et al., 1993; Zhou et al., 2011). Suitable genetic resources for breeding towards the changed climate should be pin pointed from experiments mimicking such combined climatic scenarios with several factor manipulated simultaneously.

In this study, the effects of constantly elevated temperature, [CO_2] and [O_3] as single factors, as well as the effect of the two-factor treatment of elevated temperature and [CO_2] were tested on 138 spring barley accessions in their entire life cycle. The grain yield, number of grains, amounts of ears and aboveground vegetative biomass were measured and revealed large variation between the genotypes and provided a solid estimate of the climate effect on spring barley. Accessions with the potential to mitigate effects of the future environmental changes were identified from their grain yield and climatic stability.

2. Material and Methods

2.1 Plant material

The spring barley material tested consisted of 48 landraces, 32 old cultivars (1883-1974), 53 modern cultivars (1978-2013) and 5 breeder-lines. The majority of the accessions had Nordic origin, viz. Denmark, Sweden, Norway and Finland. Eight of the modern cultivars and 22 of the landraces had non-Nordic origin (e.g. Afghanistan, Belgium, Croatia, France, Germany) and 8 accessions had unknown origin (Appendix 1). Modern cultivars and breeder-lines were supplied by the Nordic breeders in the network 'Sustainable primary production in a changing climate' (NordForsk); a few cultivars were from the BAR-OF project (ICROFS, Denmark). All other accessions were supplied by the Nordic Genetic Resource Center (NordGen; http://www.nordgen.org/).

2.2 RERAF, technical description

All plants were cultivated within the RERAF phytotron (Risø Environmental Risk Assessment Facility) at the Technical University of Denmark. RERAF consists of six identical gas-tight chambers (width 6 m, depth 4 m, height 3 m). The chambers are physically separated and with individual control of light, temperature, humidity and gasses (chamber atmospheres) and with continuous monitoring of all parameters. Within each chamber two wind turbines are placed on opposite sides to ensure air mixing. Humidity is generated by a humidifier (HumiDisk 65, Carel) placed in front of a wind turbine. The artificial light is supplied by 28 high-pressure mercury (1000 W or 400 W) and 14 halogen (250 W) lamps per chamber. The lamps can be turned on or off individually, which make it possible to simulate sunrise and sunset. The [CO_2] was applied separately in each chamber, supplied by Air Liquide Danmark A/S and the application controlled by the continuous measurements. The [O_3] in the chambers was supplied by UV Pro 550A (Crystal air products & services, Canada) generators, which were manually adjusted. Further details on RERAF are given by Alemayehu et al. (2013) and Frenck et al. (2011).

2.3 Growth conditions

Twelve seeds of each accession were sown and seedlings thinned to eight plants per pot. Pots with a volume of 11 L were filled with 4 kg of sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S, Denmark) supplemented with 10 g NPK fertilizer (21-3-10, Yara). The pots were placed on wheeled plant-tables, and plants were grown for their entire lifecycle – from seed to mature plant – in the climate phytotron RERAF with corresponding treatment. The applied climatic factors included temperature, [CO_2] and [O_3] as single factors, and a two-factor treatment with elevated temperature and [CO_2] combined. The climatic factors of the treatments were manipulated to the predicted levels of the Nordic climate ultimo 21st century. Also an ambient treatment (control) was applied mimicking present South Scandinavian climate. Table 1 shows target and experimental values.

Amount of water applied was identical for all treatments and pots. Watering was carried out by a surface dripping system that delivered 4.4 L m^{-2} day^{-1} at the beginning of the daytime regime. Water was supplied above the average precipitation of Southern Scandinavia to compensate for the higher loss of water, root distribution and drainage dictated by the pot setup. When the accessions reached maturity at Zadoks Growth Stage (ZGS) 90, watering was reduced in a stepwise fashion and ended at ZGS 99 (Zadoks et al., 1974). Watering was ended 104 days after sowing in the two-factor treatment with elevated temperature and [CO_2], 114 days after sowing in the treatment with elevated temperature, 117 days after sowing in the treatments of ambient and elevated [CO_2] and after 120 days in the treatment with elevated [O_3]. All treatments had identical artificial light and humidity conditions. The light regime was PAR (parabolic aluminized reflector) averaged at approximately 400 mol photons m^{-2} s^{-1} at canopy height (ca. 1 m), and a daily cycle of 16/8 h (day/night) with simulated sunrise and sunset within the first and last hour of light. The humidity was 55/70 % (day/night). To avoid biases of chambers, the plants were rotated weekly between and within the chambers. In practice, all chambers were set to ambient conditions before rotation; when ambient conditions were reached, the plants were moved into a new chamber and the corresponding treatment was continued. The target values of the treatments were reached within 1 h. Rotation was omitted after 97 days, as plants were too tall to be moved between chambers without damage. At any given time, the positions of the accessions were identical in all treatments.

2.4 Production parameters and data treatment

Plants were harvested individually at maturity and dried for a minimum of three weeks before threshing and then stored at constant low temperature (7°C). The production parameters, grain yield, aboveground vegetative biomass and total number of ears were noted for the individual plants. From the pooled grains of an accession in one treatment, the total number of grains was obtained by dividing with the weight of an enumerated sub-sample. Statistical calculations and figures have been performed in R version 2.15.3 (R core team, 2013), when nothing else is stated. AOT40 was measured according to Fuhrer et al. (1997):

$$AOT40_{measured} = \Sigma_i \, max(0, (C_i - 40)) \quad [1]$$

where C_i is the hourly mean ozone concentration in ppb averaged over all hourly values measured in the daylight hours (in this case 16 hours) each day and for the central 90 days of the duration of the experiment.

2.5 Stability analysis

A static yield stability index was calculated according to environmental variance (S^2; Roemer, 1917):

$$S^2_i = \sum (R_{ij} - m_i)^2 / (e - 1) \qquad [2]$$

where R_{ij} is the observed yield of the accession in the treatment j, m_i is mean yield of the accession across treatments, and e is number of environments. A dynamic yield stability index was calculated according to Wricke's ecovalence (W^2; Wricke, 1962):

$$W^2_i = \sum (R_{ij} - m_i - m_j + m)^2 \qquad [3]$$

where R_{ij} is the observed yield of the accession in the treatment j, m_i is mean yield of the accession across treatments, m_j is mean yield across treatment j of all accession and m is the grand mean, average of all m_i. Hence, W^2 states the stability dependent on the pool of accessions studied by taking means of all accessions (m_j and m) into account, whereas S^2 is a function of only the specific accession in question.

3. Results

3.1 Experimental values

The actual values of temperature, [O_3] and humidity within the treatments in the RERAF phytotron strongly corresponded to the target values (Table 1). The experimental level of [CO_2] was increased with 15-16 % in the treatments with the target value of 385 ppm. Carbon dioxide released from soil and plant respiration is the possible reason for the elevated [CO_2]. However, the higher target values of [CO_2] were in agreement over all three treatments with ambient [CO_2]. The experimental [O_3] level resulted in an AOT40 of 113 ppm/hour.

3.2 Overall effects of treatments

An example of the visually observed effect of the climate treatments on the plant phenotype is shown in Fig. 1. Elevated temperature and [CO_2] had opposite effects, as temperature decreased and [CO_2] increased height and vigour. In the two-factor treatment with combined elevated temperature and [CO_2], plant height and vigour were visually similar to those of the ambient treatment, but plants from the combined treatment showed increased development, as maturity was reached earlier. During the growth cycle elevated [O_3] caused no visual changes in plant height and vigour (Fig. 1b), but occasionally brown spots were observed.

Overall grain yield was strongly affected by the climate treatments and found to decrease 28.9 % under the two-factor climate scenario of elevated temperature and [CO_2] ($p < 0.001$; Fig. 2a). As for the single factor treatments, elevated temperature decreased overall grain yield by 55.8 % compared to ambient, and elevated [CO_2] increased the yield by 16.1 % ($p < 0.001$; Fig. 2a). Elevated [O_3] was found to decrease grain yield by 14.9 % ($p < 0.001$) but number of grains with only 4.4 % (Fig. 2a, c). The average number of grains per accession followed the pattern of grain yield for the different treatments (Fig. 2a, c). Number of ears was increased for all treatments compared to ambient ($p < 0.001$ and $p < 0.01$; Fig. 2d). Overall aboveground vegetative biomass was decreased in the single factor treatment of elevated temperature, and increased under elevated [CO_2] as single factor. However, the overall aboveground vegetative biomass was maintained under elevated [O_3] and the two-factor treatment (Fig. 2b).

Dividing the accessions into groups of cultivars and landraces revealed that the cultivar-group produced significantly more ears in all treatments ($p < 0.001$ and $p < 0.01$), except for the two-factor treatment (Table 2). In the +5°C treatment the group of cultivars produced 37.3 % more ears than the group of landraces. An increase of 11.5 % ($p < 0.05$) was found from elevated [CO_2] on overall grain yield of the cultivar-group, but not on aboveground vegetative biomass, when compared to the landrace-group. Within the cultivar group low correlation (0.04) was found between grain yield and time of release at elevated [CO_2] (data not shown). The climate effects on grain yield, number of grains, aboveground vegetative biomass and number of ears can be seen for all accessions under all treatments in a 'heat map' in Appendix 2.

3.3 Accession specific effects

The majority of accessions grouped together in the three-dimensional scatterplot of grain yield under elevated temperature, [CO_2] and their combination relative to grain yield under ambient conditions (Fig. 3). Three accessions, 'Sanglich', 'Lantkorn från Jämtland' and 'Fabel Sejet' were outliers as they were apparently highly productive in the future climate scenarios, however they ranked as the three least productive accessions in the ambient treatment. In addition to these three accessions, 17 accessions had grain yields that were reduced less than 10 % in the two-factor treatment; they were 'Alliot', 'Justus', 'Bor05135', 'Brage', 'Brio', 'Fairytale', 'Griechische', 'Gunnar', 'Jacinta', 'Kushteki', 'Lysimax', 'Moscou', 'NOS 17009-53', 'Calisi', 'Oslo', ‘Sebastian’ and ‘Sort Glatstakket’. The positive effect of elevated [CO_2] caused an above 50 % increase in grain yield of the accessions ‘Alliot’, ‘Brage’, ‘Fairytale’, ‘Gunnar’, ‘Jacinta’, ‘Manschurei’ and ‘Odin’. Elevated [CO_2] was found to be the factor that increased grain yield, aboveground vegetative biomass and number of grains of most accessions. Seven accessions were found with less than 30 % decreased grain yield under elevated temperature (+5°C) and they were ‘Evergreen’, ‘Fræg’, ‘Königsberg’, ‘Luusua’, ‘Odin’, ‘Sebastian’ and ‘Sort Glatstakket’. Variance in grain yield to elevated [O_3] was found within the group of landraces, old and modern cultivars as well as breeder-lines (Fig. 4). The group of landraces apparently showed the largest variation in grain yield response relative to ambient under elevated [O_3. The accessions ‘Agneta’, ‘Juli Abed’ and ‘Ylenjoki’ all produce increased grain yield under elevated [O_3].

3.4 Trends in yield stability

The calculated static environmental variance (S^2) and the dynamic Wricke’s ecovalence (W^2) of the 40 accessions with the highest averaged mean yield across treatments are listed in Table 3. Old and modern cultivars as well as landraces and a breeder-line were found within the set of the 40 accessions with highest mean yield across treatments and various scores for static and dynamic stability. On the whole set of 138 accessions S^2 spanned from 0.58 to 16.79 and W^2 from 0.27 to 33.60, where a low value indicate stability (Appendix 3). The cultivar ‘Sebastian’ was static stable and high yielding by ranking 11 for S^2 and fourth for averaged mean grain yield across treatments. Further ‘Sebastian’ ranked nine in grain yield relative to ambient in the two-factor treatment. The accession ‘Åsa’ showed the best static stability (ranked 8) within Table 3. However, ‘Åsa’ also produced an averaged mean grain yield that placed her at the 40th place. The cultivars ‘Agneta’, ‘Jacinta’ and ‘Laurikka’ were found to respond differently to either of the treatments than the majority of the accessions by a high W^2 score. Hereof ‘Agneta’ and ‘Jacinta’ ranked first and second according to averaged mean grain yield. Cultivars identified to be static stable but with low mean grain yield were e.g. ‘Moscou’, ‘Calisi’, ‘Oslo’, and ‘Sort Glatstakket’ (Appendix 3).

4. Discussion

Breeding resilient cultivars, which can meet the increased demand for food and feed in the future, is jeopardized by several unknown environmental challenges and genetic limitations. Some of the open questions relates to the speed of climate change, the frequency of extreme weather events and whether sufficient genetic resources are available to mitigate the climate change effect on grain yields. Modelling studies and selection experiments indicate that naturally occurring microevolution of crops is likely to be too slow to keep up with the pace of climate change (Alemayehu et al., 2013; Rosenzweig and Parry, 1994; Svenning and Sandel, 2013). The possibility that climate change occur too rapidly for adaptation to keep up, adds to the need for identification of exploitable genetic resources for stable and high yielding cultivars for the future.

4.1 Stable and high yielding cultivars for the future climate

In this study static S^2 and dynamic W^2 of a high number of accessions under climatic factors at expected future levels are reported for the first time (Table 3, Appendix 3). Stability has traditionally been emphasized in subsistence farming as local stability being crucial for survival (Sinebo, 2005; Annicchiarico, 2002). However, with climate change progressing fast we can, within a relative short timeframe, face a situation in the Nordic countries where our elite cultivars must possess local stability. Therefore emphasis on stability in combination with high grain yield must be prioritized to ensure the production of the future (Becker and Leon, 1988; Powell et al., 2012; Sinebo 2005). We found the Danish cultivar 'Sebastian' is a candidate exhibiting high static stability combined with high yield. This is in agreement with 'Sebastian' being used in very diverse climates spanning from Ukraine through south-western Europe and Chile. Further, 'Sebastian' has through breeding given rise to the successful cultivar 'Quench'. 'Quench' also demonstrates broad environmental adaptation and high yield (R. Hjortshøj, Sejet Plant Breeding, pers. comm.). Here we found that an accession demonstrating broad adaptation to the climates of today also showed static stable under future levels of climatic factors. This emphasise that static stability should be used as tool to breed climate resilient cultivars for the future.

The Swedish cultivar 'Åsa' from 1949 was found to be static stable though not as high yielding as 'Sebastian'. Crossing 'Åsa' with a high yielding modern accession such as 'Jacinta', with great response to elevated [CO_2] found by the high dynamic stability score, is an approach to the development of climate resilient cultivars. The knowledge on S^2 and W^2 of the accessions of this study combined with knowledge present in plant breeding companies should be exploited e.g. the accession ‘Agneta’ to introduce tolerance to elevated [O_3], ‘Luusua’ to mitigate negative effect of elevated temperature and opposite ‘Oslo’ to introduce static stability and so on. Breeding is genetics and by no means a simple art, but widening it to focus on climate resilience with stability we argue is about time and possible.

4.2 Effects of single- and two-factor treatments

Published enclosure, Open-top or FACE studies often only include one or few cultivars, when assessing the effects of elevated temperature, [CO_2] and/or [O_3]. This study comprised a set of 138 diverse spring barley accessions and the overall trends identified in response to the abiotic factors and therefore have strong solidity for spring barley. That temperature strongly decreases grain yield is supported by earlier findings (Alemayehu et al., 2013; Clausen et al., 2011; Högy et al., 2013), whereas it is difficult to unravel if the decrease is caused by heat or drought stress (Barnabás et al., 2008; Powell et al., 2012). In the present study, the treatments including elevated temperature have, despite that humidity was applied at the same level in all treatments, experienced an increased level of water restriction compared to the single factor treatments of elevated [CO_2] or [O_3] and the ambient treatment, due to the vapor pressure deficit. Therefore, the decreased grain yield found from the treatment of elevated temperature and the two-factor treatment is most likely caused by concerted elevated temperature and restriction in water consumption. The identified positive response in grain yield of 16 % to elevated [CO_2] was lower for the large barley set of this study than previously reported for considerable less accessions in enclosure studies (Alemayehu et al., 2013; Clausen et al., 2011; Kimball, 1986), but in agreement with the FACE study of Weigel and Manderscheid (2012) on winter barley. The very variable and large set of accessions analysed in the present study should provide a solid basis for general statements on spring barley. Concerning the effect of elevated [O_3] the decrease in grain yield (15 %) found under conditions with AOT40 at 113 ppm/hour is in agreement with spring barley previously reported to tolerate high [O_3] e.g. spring barley can tolerate a 25 times higher dose than wheat (Mills et al., 2007). However, the current data set indicates a relative higher sensitivity to ozone than reported by Mills et al. 2007. In the two-factor treatment, the negative effect on grain yield by elevated temperature was not counter-balanced by the positive effect of elevated [CO_2] and grain yield decreased 29 %. Such counter-balance of grain yield has been hypothesised (Tubiello et al., 2007), but its absence is also supported by Hakala (1998) and Clausen et al. (2011). Considering that effects to grain yield of the single factors temperature and [CO_2] were not found to be additive in their combined treatment, we argue that response in complex environments cannot easily be depicted from single factor scenarios, also emphasised by Atkinson and Urwin (2012). When searching for genetic resources exploitable for production under future climate scenarios, where numerous abiotic factors are in play, it must be considered, how the results from screens performed under single factor treatments can be used. It might even be questioned, if the traditional set up of testing for tolerance to single factors is worth the investment, when breeding is the first aim.

In the treatments including constantly elevated temperature as single factor or combined with elevated [CO_2], the total number of ears increased, whereas number of grains decreased. The impaired grain formation can be caused by the elevated temperature disturbing several developmental steps throughout the lifecycle of the plant. Heat exposure prior to anthesis has previously been found to reduce the number of florets and subsequently the number of grains, whereas heat exposure at anthesis aborts the florets at the primordial stage (Rajala et al., 2011; Ugarte et al., 2007). During the grain filling stage grain weight and also size is defined, and heat exposure has been found to decrease both (Rajala et al., 2011; Ugarte et al., 2007). To maintain grain yield under elevated temperature breeding for early heading could potentially secure a sufficient period for grain filling, as it is suggested in wheat (Tewolde et al., 2006).

4.3 Identification and exploitation of genetic resources for the future

The accessions found in the present study with a high grain yield and stability are bred in different countries over a large time span, indicating that genetic resources for climate resilience are available from diverse sources. The best genotypes of this study e.g. ‘Sebastian’, ‘Griechische’, ‘Brio’, ‘Jacinta’ and ‘Kushteki’ should be exploited in breeding programmes. Still, the introduction of new germplasm in breeding programmes needs strong arguments, while it is doubtful if results from non-natural environments are considered fully adequate by breeders. Despite the large phytotron chambers (24 m^2) used in the present study, the authors acknowledge that the environment within the RERAF phytotron with constant day/night-temperature, no clouds, pot set up and limited biotic stress is non-natural. Also, results are given relative to an ambient treatment that for some accessions might be far from their normal ambient environment. Phytotron experiments though have the advantage of minimizing the effects of changes in factors such as water application and humidity leading to considerable less variation than found in field studies. Further, it is possibly to increase [CO_2] at a lower cost within a phytotron and increase the temperature considerable. FACE experiments, where [CO_2] is elevated, are a natural next-step from the phytotron experiments. Generally, the positive effects of [CO_2] on production from FACE studies have been found to be lower than the effect found in enclosure studies. In that context, it has been debated if enclosure studies have thrown a too optimistic light on the future plant production and food security, when results from enclosure studies have been incorporated in models and used for predictions (Long et al., 2006; Tubiello et al., 2007; Wang et al., 2013; Ziska and Bunce, 2007). In this study we identified that the effect of elevated [CO_2] is altered under concurrently elevated temperature, why multifactor studies are the most reliable for forecasting the effects of future environmental changes. However, the methods of roof technology or the infrared reflectance technology used to conduct elevated temperature under field-conditions has up to today not proven capable to reach realistic elevated temperature regimes or resulted in asymmetric warming (e.g. Bruhn et al., 2013; Kimball et al. 2008).

4.4 Productivity in cultivars and landraces

Considering that breeding for generation has aimed at increased grain yield, it is surprising that the group of cultivars only under elevated [CO_2] produced a higher grain yield than the group of landraces. Since the group of cultivars outperformed the group of landraces just under increased [CO_2], one could assume that enhanced net-photosynthesis to elevated [CO_2] unconsciously has been targeted through breeding. However, the effect was not found for aboveground vegetative biomass. Within the cultivar-group, no continuously improved grain yield from elevated [CO_2] was found in time, which is in accordance with previous studies by Manderscheid and Weigel (1997) and Ziska et al. (2004). In regard to amount of ears produced, the group of cultivars consistently produced significantly (p > 0.001 and p > 0.01) more ears than landraces in all single factor treatments. Hence, the physiological aptitude for increased grain yield in cultivars is present, but the grains were only formed under elevated [CO_2] conditions. The present study revealed that the group of cultivars and group of landraces did not perform significantly different in the two-factor treatment, which was the most realistic future climate scenario with the combination of elevated temperature and [CO_2]. Therefore, it seems meaningful to search for genetic resources for future compounded environments among old as well as modern germplasm. The drawback from use of landraces such as 'Kushteki' or 'Oslo' in breeding programmes is the risk of transferring unwanted genetic material, and disturbing established allele complexes in the optimized germplasm. Nevertheless, introduction of germplasm from exotic material can be advantageous in widening the genetic base of crops (Brantestam et al., 2007; Malysheva-Otto et al., 2007; Russel et al., 2000) in addition to the introduction of identified valuable allele(s). Traditionally, disease resistance genes have been found in exotic material and successfully transferred to elite cultivars (Colton et al., 2006; Czembor, 2000; Silvar et al., 2013). We encourage that this approach should be expanded to include genes providing tolerance to climatic stress to secure the production under future climate conditions.

5. Conclusion

For the first time a comprehensive set of 138 spring barley accessions has been screened over their entire life cycle under a two-factor climate scenario of elevated [CO_2] and temperature. Further, static and dynamic stability of grain yield over five different climate treatments were reported for the 138 accessions. Our results show an overall substantially decreased grain yield of 29 % in the two-factor climate scenario with 700 ppm [CO_2] and a temperature increase of 5°C. Effects of the single factors of the two-factor treatment as well as elevated [O_3] was also identified. The substantial decrease in production found under a realistic future climate scenario (700 ppm CO_2, +5°C) accentuates the great challenge plant breeding faces. Our results emphasises the need for phenotyping of plants under realistic multifactor climate conditions, as single factor experiments might provide limited or even misleading information to the forecasting of changes and strategies for mitigation. In this study we have identified potential genetic resources from the production performance and stability, that can mitigate the production loss in future climate scenarios. These genetic resources could and should be exploited in breeding programmes.

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Appendix A. Supplementary data

This material is available in print in the dissertation and digitally on the attached CD-rom.

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Table 1
Target and experimental levels of manipulated climatic factors of applied treatments.

Treatment	**Temp** (day/night)		CO_2 (constant)		O_3 (constant)		**humidity** (day/night)	
	target	experimental	target	experimental	target	experimental	target	experimental
ambient	19/12°C	18.9±1.2/11.8±0.8	385 ppm	448.5±81.1	0	1.4±1.4	55/70	55.7±2.5/69.9±1.5
+ CO_2	19/12°C	19.0±1.2/12.5±2.1	700 ppm	684.7±41.1	0	0.98±1.7	55/70	55.3±5.1/69.4±5.9
+ temp	24/17°C	23.9±1.4/16.8±0.8	385 ppm	448.4±74.4	0	1.9±1.2	55/70	55.9±2.8/69.8±1.6
+ temp & CO_2	24/17°C	23.8±1.3/16.9±0.9	700 ppm	688.3±38.2	0	1.5±1.4	55/70	56.0±2.9/69.8±1.8
+ O_3	19/12°C	18.9±1.2/11.9±1.0	385 ppm	443.1±67.5	100-150 ppb	121.1±32.8	55/70	55.7±2.4/69.8±1.7

Table 2. Difference (%) between the group of cultivars to the group of landraces for averaged production parameter values. Negative values indicate that the production of the cultivar group is less than that of the landrace group. Significances: 0.001 : ***; 0.01 : **; 0.05 : *.

	ambient	tmp & $[CO_2]$	$[CO_2]$	tmp	$[O_3]$
No of ears	19.7**	7.4	23.7***	37.3***	23.5***
Grain yield	4.0	2.9	11.5*	13.3	3.5
Aboveground vegetative biomass	-5.0	-6.6	-3.0	9.5	-5.0
No of grains	-4.8	-6.6	- 2.5	4.5	-5.3

Table 3. Environmental variance (S^2) and Wricke's ecovalence (W^2) over 5 climate treatments for 40 accessions. The 40 accessions are the one with highest averaged mean yield across treatments (m_i) of the 138 accessions; rank given in ( ). Low value indicates strong stability. CV: cultivar, BL: breeder-line, LR: landrace.

Accession name	NGB no.	Culton type	Year of release	m_i	S_i^2		W_i^2	
Agneta	NGB1508	CV	1978	7.84	7.43	(113)	19.98	(134)
Jacinta	NGB16665	CV	1999	7.74	16.79	(138)	33.6	(138)
Linus	NGB13482	CV	1997	7.52	7.41	(112)	4.69	(72)
Sebastian		CV	2002	7.36	1.35	(11)	4.34	(62)
Laurikka		CV	2012	7.26	8.57	(122)	10.8	(116)
Paavo	NGB13661	CV	1959	7.15	5.72	(90)	2.99	(40)
Griechische KVL 56	NGB9333	LR		7.12	5.09	(80)	4.4	(65)
Odin	NGB16755	CV	1981	7.10	4.42	(65)	5.92	(90)
Stange	NGB2109	CV	1978	7.05	7.36	(111)	3.71	(51)
Evergreen		CV	2010	7.04	3.85	(52)	2.59	(37)
Columbus		CV	2009	7.03	11.55	(132)	10.75	(115)
Brio	NGB9327	CV	1924	7.02	6.13	(95)	5.83	(87)
Gaute	NGB16732	CV	2000	6.97	6.32	(102)	2.18	(28)
Manschurei	NGB9624	LR		6.97	11.97	(136)	13.45	(127)
Amalika		CV	2012	6.94	4.88	(75)	5.56	(83)
Iron		CV	2007	6.89	3.44	(42)	1.72	(19)
Alliot	NGB16757	CV	1999	6.88	4.33	(63)	3.97	(54)
Szeged KVL 347	NGB9478	LR		6.84	4.29	(61)	4.84	(79)
Danpro	NGB9659	CV	1969	6.82	11.58	(133)	9.33	(110)
Orthega		CV	1997	6.81	11.15	(131)	10.95	(117)
Caruso	NGB15059	CV	1991	6.78	3.85	(51)	0.93	(6)
Møyjar	NGB2106	CV	1969	6.76	8.19	(116)	4.36	(63)
Brage		CV	2010	6.69	6.48	(106)	4.71	(73)
Bjørne	NGB9326	LR		6.64	6.81	(107)	3.57	(49)
Freja	NGB1485	CV	1941	6.64	4.83	(72)	11.75	(120)
Hannuksela	NGB325	LR		6.58	5.77	(92)	3.04	(42)
Prominant	NGB15066	CV	1999	6.51	6.37	(104)	5.86	(88)
Bor09801		BL		6.49	2.80	(30)	13.85	(129)
Peruvian	NGB8880	LR		6.48	3.35	(40)	1.66	(18)
Birka	NGB4712	CV	1981	6.47	4.72	(69)	1.59	(15)
Kushteki K.77	NGB6288	LR		6.46	5.29	(83)	5.79	(86)
Sarkalahti ME0103	NGB27	LR		6.44	9.92	(127)	7.51	(102)
Lysimax	NGB15055	CV	1994	6.40	2.77	(28)	1.97	(25)
Trekker		CV	2013	6.39	1.36	(12)	4.24	(59)
Grenoble I KVL 131	NGB9378	LR		6.35	4.15	(59)	1.54	(13)
Metz KVL 124	NGB9373	LR		6.30	1.97	(17)	8.49	(107)
Prestige	NGB16750	CV	2000	6.27	3.71	(48)	1.55	(14)
Cicero	NGB16756	CV	1999	6.27	7.98	(115)	4.51	(67)
Pavia KVL 386	NGB9501	LR		6.24	8.22	(119)	7.98	(104)
Åsa	NGB4640	CV	1949	6.23	1.15	(8)	8.75	(108)

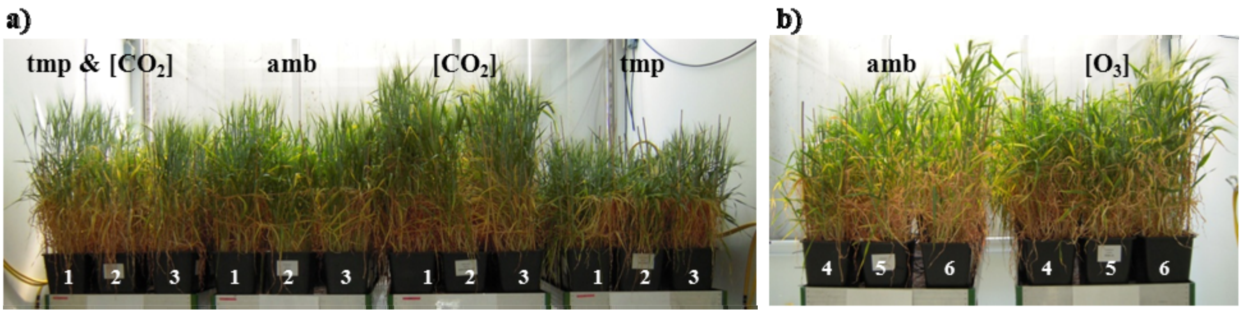


Fig. 1. Visual comparison of spring barley grown under five climate treatments. a) from left to right with blocks of three cultivars (1: 'Lysimax', 2: 'Paavo', 3:'Linus'): elevated [CO_2] and temperature in combination (tmp & [CO_2]), ambient (amb), elevated [CO_2] ([CO_2]), elevated temperature (tmp). b) from left ambient (amb) and elevated [O_3] ([O_3]) with block of three cultivars (4: 'Alf', 5: 'Fløya', 6: 'Åsa').

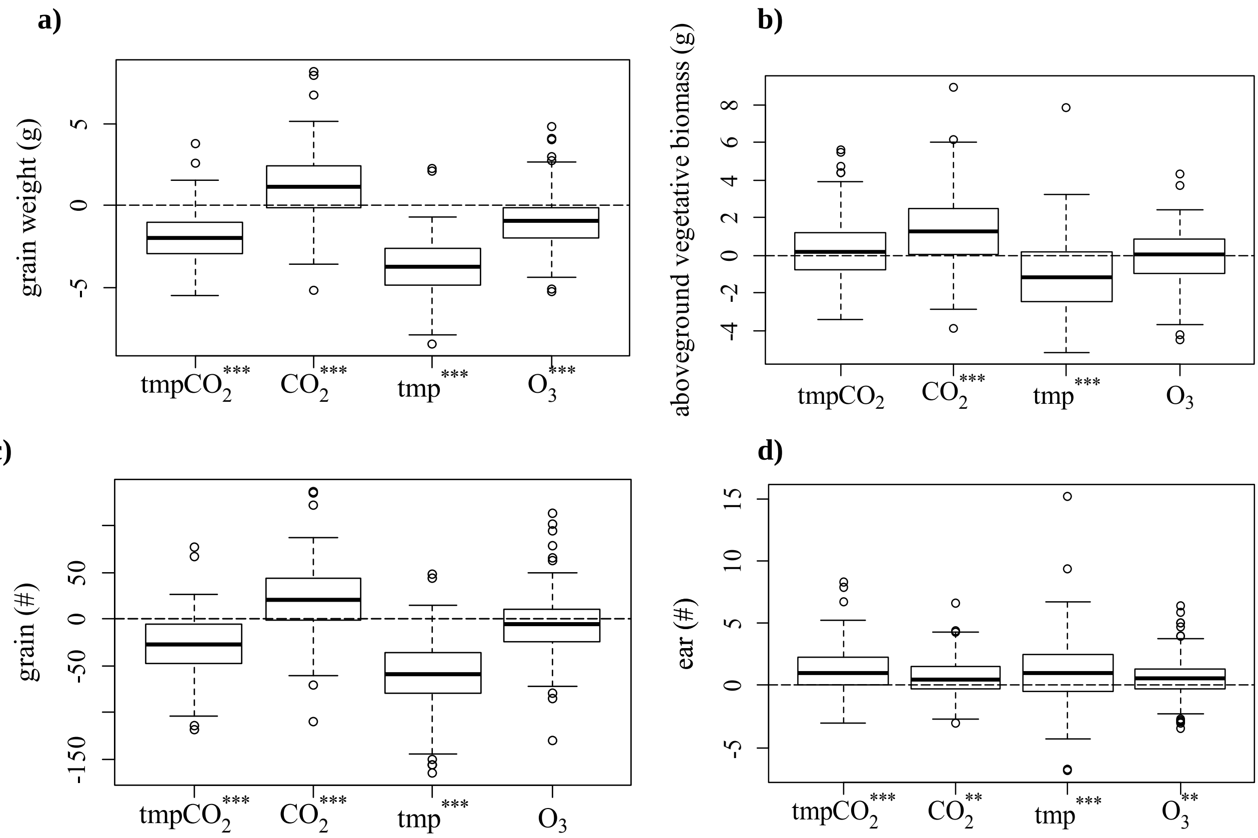


Figure 2. Treatment effects on 138 spring barley accessions (average g/plant) grown under 4 climate treatments. Significance of the treatments to ambient according to T-test. Significance codes: 0.001 = ***; 0.01 =**; 0.05 =*. **a**) Grain yield **b**) Above-ground vegetative biomass **c**) number of grains **d**) number of ears. Dashed line: production in the ambient control treatment (0). Circles represent the average of eight plants of an accession. Median indicated in bold and whiskers gives quartile group 1 and 4.

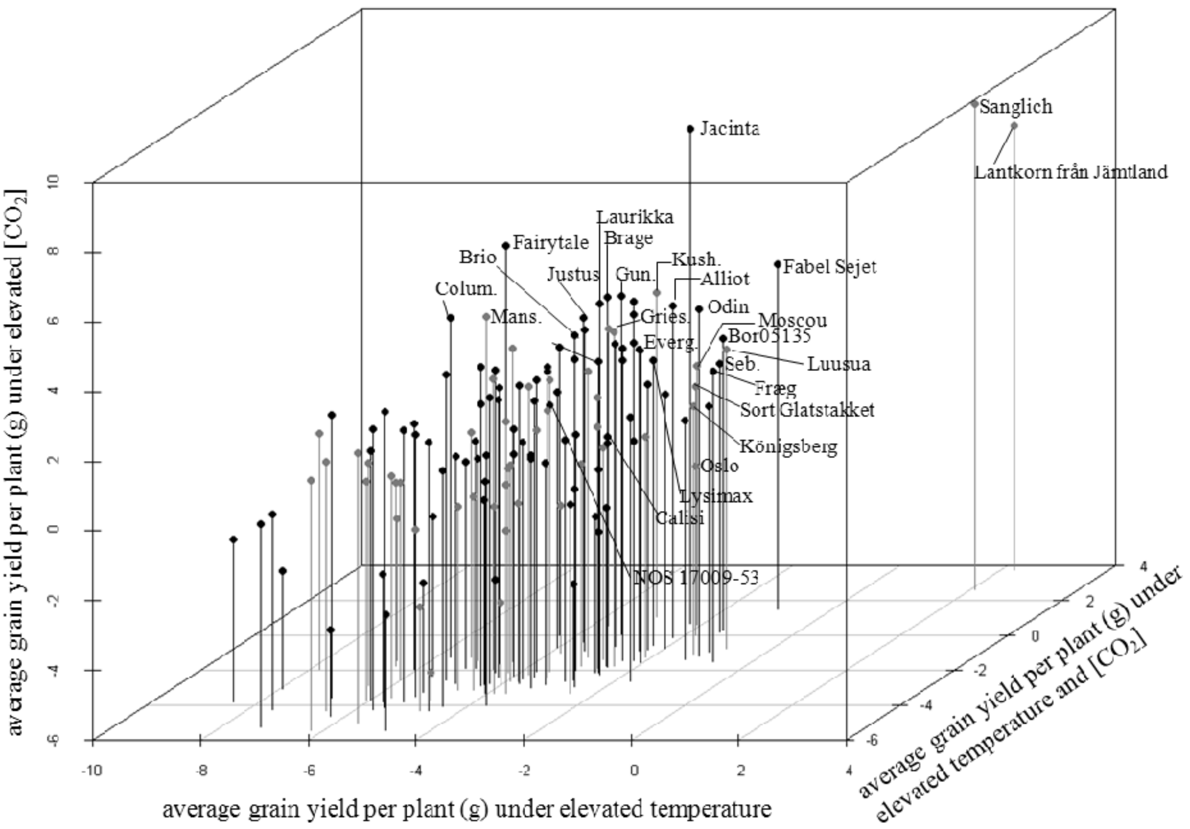


Figure 3. Grain yield relative to ambient of 138 spring barley accessions cultivated under elevated [CO_2] and temperature as single factors and their combination. Black points are cultivars or breeder-lines and grey are landraces. Caru.: Caruso, Colum.:Columbus, Everg.: Evergreen, Fab.: Fabel Sejet, Fair.: Fairytale, Gun: Gunnar, Gries.: Grieschische, Kush.: Kushteki, Laur.: Laurikka, Mans.: Manschurei, Seb.: Sebastian.

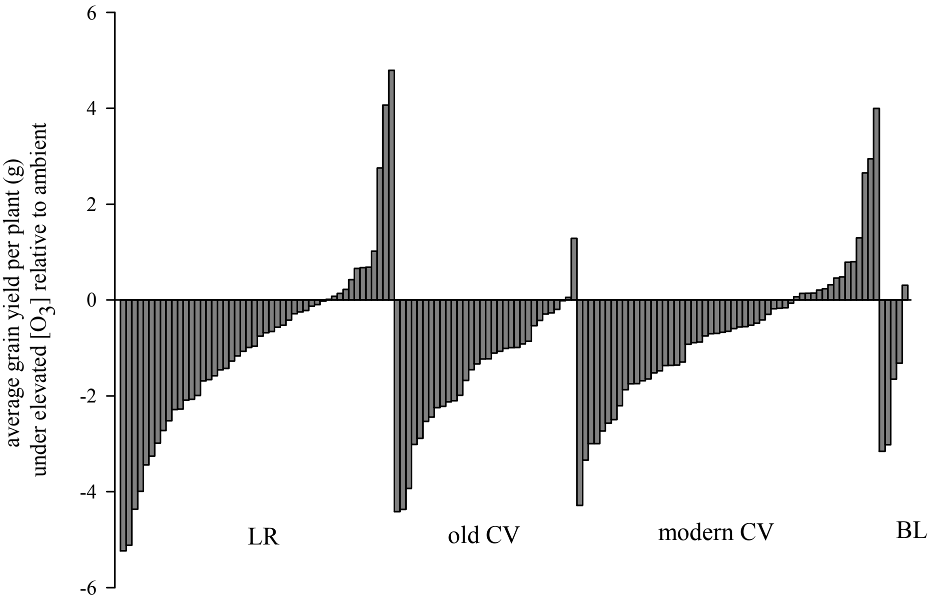


Figure 4. Grain yield relative to ambient of 138 spring barley accessions cultivated under elevated [O_3]. LR: landraces, old CV: cultivars from 1883-1974, modern CV: cultivars from 1978-2013 and BL: breeder-lines.

Supplementary material

Manuscript 1

APPENDIX 1

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Bor05135	Bor05135		BL	Finland	2		Boreal Plant Breeding
Bor09801	Bor09801		BL	Finland	6		Boreal Plant Breeding
NOS 15251-52	NOS 15251-52		BL	Denmark	2		Nordic Seed A/S Plant Breeding
NOS 16140-51	NOS 16140-51		BL	Denmark	2		Nordic Seed A/S Plant Breeding
NOS 17009-53	NOS 17009-53		BL	Denmark	2	expected 2014	Nordic Seed A/S Plant Breeding
Agneta	Agneta	NGB1508	CV	Sweden	6	1978	NordGen
Alabama	Alabama		CV	Germany	2	1999	BAR-OF project (ICROFS,Denmark)
Alf	Alf	NGB4707	CV	Denmark	2	1978	NordGen
Alliot	Alliot	NGB16757	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Amalika	Amalika		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Anakin	Anakin		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Anita	Anita	NGB15250	CV	Norway	6	1962	NordGen
Arla	Arla	NGB2681	CV	Sweden	2	1962	NordGen
Arve	Arve	NGB11311	CV	Norway	6	1990	NordGen
Åsa	Åsa	NGB4640	CV	Sweden	6	1949	NordGen
Birgitta	Birgitta	NGB1494	CV	Sweden	2	1963	NordGen
Birka	Birka	NGB4712	CV	Sweden	2	1981	NordGen
Brage	Brage		CV	Norway	6	2010	Graminor Plant Breeding
Brazil	Brazil		CV	France	2	2000	BAR-OF project (ICROFS,Denmark)
Brio	Brio	NGB9327	CV	Sweden	6	1924	NordGen
Calisi	Calisi		CV	Denmark	2	2013	Nordic Seed A/S Plant Breeding
Carlsberg	Carlsberg	NGB9442	CV	Denmark	2	1946	NordGen
Caruso	Caruso	NGB15059	CV	Denmark	2	1991	NordGen
Chevallier Tystofte	Chevallier Tystofte	NGB9443	CV	Denmark	2	1883	NordGen
Cicero	Cicero	NGB16756	CV	Denmark	2	1999	BAR-OF project (ICROFS,Denmark)
Columbus	Columbus		CV	Denmark	2	2009	Sejet Plant Breeding I/S
Culma	Culma		CV	Belgium	2	1997	BAR-OF project (ICROFS,Denmark)
Danpro	Danpro	NGB9659	CV	Denmark	2	1969	NordGen
Danuta	Danuta		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Denso Abed	Denso Abed	NGB8826	CV	Denmark	2	before 1965	NordGen
Drost Pajbjerg	Drost Pajbjerg	NGB6281	CV	Denmark	2	1951	NordGen
Edvin	Edvin		CV	Finland	6	2008	Boreal Plant Breeding

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Elmeri	Elmeri		CV	Finland	6	2009	Boreal Plant Breeding
Etu	Etu	NGB2679	CV	Finland	6	1970	NordGen
Eva	Eva	NGB1502	CV	Sweden	2	1973	NordGen
Evergreen	Evergreen		CV	Denmark	2	2010	Nordic Seed A/S Plant Breeding
Fabel Sejet	Fabel Sejet		CV	Denmark	2	2001	BAR-OF project (ICROFS,Denmark)
Fairytale	Fairytale		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Fero	Fero	NGB8231	CV	Denmark	2	1943	NordGen
Fløya	Fløya	NGB4664	CV	Norway	6	1939	NordGen
Fræg	Fræg	NGB8860	CV	Norway	6	1948	NordGen
Freja	Freja	NGB1485	CV	Sweden	2	1941	NordGen
Galant Carlberg	Galant Carlberg	NGB6308	CV	Denmark	2	1985	NordGen
Gaute	Gaute	NGB16732	CV	Norway	6	2000	Graminor Plant Breeding
Gunnar	Gunnar	NGB1515	CV	Sweden	2	1981	NordGen
Hafnia	Hafnia	NGB8878	CV	Denmark	2	1958	NordGen
Hannchen	Hannchen	NGB4617	CV	Sweden	2	1902	NordGen
Harbinger	Harbinger		CV	Finland	2	2009	Boreal Plant Breeding
Harriot	Harriot		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Helium	Helium		CV	Denmark	2	2001	Nordic Seed A/S Plant Breeding
Hydrogen	Hydrogen	NGB20346	CV	Denmark	2	2000	Nordic Seed A/S Plant Breeding
Ida	Ida	NGB2672	CV	Sweden	2	1979	NordGen
Iron	Iron		CV	Denmark	2	2007	Nordic Seed A/S Plant Breeding
Jacinta	Jacinta	NGB16665	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Jadar II	Jadar II	NGB457	CV	Norway	6	1947	NordGen
Jotun	Jotun	NGB4618	CV	Norway	6	1930	NordGen
Juli Abed	Juli Abed	NGB4585	CV	Denmark	6	1909	NordGen
Justus	Justus		CV	Finland	6	2013	Boreal Plant Breeding
Karin	Karin	NGB13021	CV	Sweden	6	1988	NordGen
Karri	Karri	NGB287	CV	Finland	2	1967	NordGen
Landora	Landora		CV	Germany	2	2000	BAR-OF project (ICROFS,Denmark)
Laurikka	Laurikka		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Lavrans	Lavrans	NGB16727	CV	Norway	6	1999	Graminor Plant Breeding
Linus	Linus	NGB13482	CV	Sweden	2	1997	NordGen
Lysimax	Lysimax	NGB15055	CV	Denmark	2	1994	NordGen
Mari	Mari	NGB1491	CV	Sweden	2	1960	NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Møyjar	Møyjar	NGB2106	CV	Norway	2	1969	NordGen
Nord	Nord	NGB9942	CV	Finland	6	1988	NordGen
Odin	Odin	NGB16755	CV	Denmark	2	1981	NordGen
Orthega	Orthega		CV	Germany	2	1997	BAR-OF project (ICROFS,Denmark)
Otira	Otira	NGB16752	CV	Denmark	2	1997	BAR-OF project (ICROFS,Denmark)
Paavo	Paavo	NGB13661	CV	Finland	6	1959	NordGen
Pallas	Pallas		CV	Sweden	2	1970	BAR-OF project (ICROFS,Denmark)
Piikkiönohra	Piikkiönohra	NGB8234	CV	Finland	2	1922	NordGen
Pinocchio	Pinocchio		CV	Denmark	2	2011	Sejet Plant Breeding I/S
Pirkka	Pirkka	NGB292	CV	Finland	6	1952	NordGen
Prestige	Prestige	NGB16750	CV	France	2	2000	The Danish AgriFish Agency, Tystofte
Prominant	Prominant	NGB15066	CV	Denmark	2	1999	NordGen
Punto	Punto	NGB23207	CV	Denmark	2	1995	BAR-OF project (ICROFS,Denmark)
Rex Abed	Rex Abed	NGB9465	CV	Denmark	2	1913	NordGen
Sebastian	Sebastian		CV	Denmark	2	2002	NordGen
Severi	Severi		CV	Finland	6	2013	Boreal Plant Breeding
Simba	Simba		CV	Denmark	2	2002	NordGen
Stange	Stange	NGB2109	CV	Norway	2	1978	NordGen
Tammi	Tammi	NGB6925	CV	Finland	6	1937	NordGen
Tore	Tore	NGB6605	CV	Norway	6	1986	NordGen
Trekker	Trekker		CV	Finland	2	2013	Boreal Plant Breeding
Tron Sejet	Tron Sejet	NGB9655	CV	Denmark	2	1978	NordGen
Visir	Visir	NGB1496	CV	Sweden	2	1971	NordGen
Zita	Zita	NGB7236	CV	Denmark	2	1974	NordGen
Anita Högsby-korn	Anita Högsby-korn	NGB263	L	Sweden	2		NordGen
Bjørne	Bjørne	NGB9326	L		6		NordGen
Bryssel	Bryssel KVL 28	NGB8222	L	Belgium	6		NordGen
Cluj	Cluj KVL 100	NGB9355	L	Romania	6		NordGen
Dønnes	Dønnes	NGB9448	L	Norway	6		NordGen
Gardez Pandshir	Gardez Pandshir K.173	NGB4669	L	Afghanistan	6	194811--	NordGen
Grenoble	Grenoble I KVL 131	NGB9378	L	France	6		NordGen
Griechische	Griechische KVL 56	NGB9333	L	Greece	6		NordGen
Hannuksela	Hannuksela	NGB325	L	Finland	6		NordGen
Junkkari	Junkkari	NGB307	L	Finland	6		NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Kilpau	Kilpau ME0201	NGB1159	L	Finland	6	19810819	NordGen
Königsberg	Königsberg KVL 18	NGB9310	L		6		NordGen
Kushteki	Kushteki K.77	NGB6288	L	Afghanistan	6	1948----	NordGen
Langaks	Langaks	NGB6300	L	Faroe Islands	6	196108--	NordGen
Lantkorn från Jämtland	Lantkorn från Jämtland	NGB6927	L	Sweden	6		NordGen
Laukko	Laukko	NGB16881	L	Finland	2		NordGen
Ljubljana KVL 15	Ljubljana KVL 15	NGB9308	L	Croatia	6		NordGen
Ljubljana KVL 395	Ljubljana KVL 395	NGB6941	L	Croatia	2		NordGen
Luusua	Luusua EH0401	NGB792	L	Finland	6	19790907	NordGen
Lynderupgaard	Lynderupgaard	NGB9529	L	Denmark	6	195205--	NordGen
Magdeburg	Magdeburg KVL 358	NGB9485	L	Germany	2		NordGen
Manschurei	Manschurei	NGB9624	L		6		NordGen
Metz	Metz KVL 124	NGB9373	L	France	6		NordGen
Montpellier	Montpellier KVL 209	NGB9410	L	France	2		NordGen
Moscou	Moscou KVL 353	NGB9482	L		2		NordGen
Näckte von Nepal	Näckte von Nepal	NGB9305	L	Nepal	6		NordGen
Nordslesvigsk Kæmpe	Nordslesvigsk Kæmpe	NGB9339	L	Denmark	6	1890	NordGen
Nue Grosse	Nue Grosse	NGB9436	L	Denmark	2		NordGen
Oppdal	Oppdal	NGB13670	L	Norway	6		NordGen
Osiris	Osiris J-1277	NGB9639	L		6		NordGen
Oslo	Oslo KVL 25	NGB9315	L	Norway	6		NordGen
Pavia	Pavia KVL 386	NGB9501	L	Italy	2		NordGen
Peruvian	Peruvian	NGB8880	L	Peru	6		NordGen
Probstei	Probstei/Tabor KVL 362	NGB9487	L	Czech Republic	2		NordGen
Rauto	Rauto	NGB265	L	Finland	2;6		NordGen
Rehakka	Rehakka-65	NGB314	L	Finland	2		NordGen
Sanglich	Sanglich K.128	NGB8872	L	Afghanistan	6	194807--	NordGen
Sarkalahti	Sarkalahti ME0103	NGB27	L	Finland	6	19800810	NordGen
Smolensk	Smolensk KVL 741	NGB9623	L	Russian Federation	2		NordGen
Solenbyg	Solenbyg	NGB13402	L	Norway	6		NordGen
Sort Glatstakket	Sort Glatstakket	NGB9345	L		6		NordGen
Sort Himalaya	Sort Himalaya	NGB9516	L		2		NordGen
Stjernebyg fra Færøerne	Stjernebyg fra Færøerne	NGB4701	L	Faroe Islands	6	196908--	NordGen
Szeged	Szeged KVL 347	NGB9478	L	Hungary	2		NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Tartu	Tartu KVL 349	NGB9479	L	Estonia	2		NordGen
Vilm	Vilm KVL 248	NGB9435	L	Germany	2		NordGen
Vilmorin	Vilmorin KVL 126	NGB9375	L		6		NordGen
Ylenjoki	Ylenjoki AP0301	NGB4413	L	Finland	2;6	19830825	NordGen
excluded, winter barley	Tschermaks 2 ZLG	NGB6277	L	Germany	2		
excluded, winter barley	Narvik	NGB15074	CV	Denmark		1996	

Accession name	Location / Breeder institute	Culton pedigree
Bor05135	Boreal Plant Breeding	
Bor09801	Boreal Plant Breeding	
NOS 15251-52	Nordic Seed A/S Plant Breeding	
NOS 16140-51	Nordic Seed A/S Plant Breeding	
NOS 17009-53	Nordic Seed A/S Plant Breeding	Iron x Keops
Agneta	Sveriges Utsadesforening Svalöf	(Åsa x Frisia) ^2 x (Edda II ^4 x Monte Cristo)
Alabama	KWS SAAT AG	(Ml-i x 2.51784) x Krona
Alf	Nordic Seed A/S Plant Breeding	Mutant in Bomi
Alliot	Nordic Seed A/S Plant Breeding	Waggon*Imidis
Amalika	Nordic Seed A/S Plant Breeding	NOS 1184-07 x Quench
Anakin	Sejet Plant Breeding I/S	Tumbler x Respons
Anita	Norwegian Agrcultural University	HOL-44 x Arve; / (Asplund x Ds295) x Varde
Arla	Weibullsholm Plant Breeding Institute	(Maja x ((Hanna x Svanhals) x Opal)) x Tammi
Arve	Graminor Plant Breeding	(Otra x Vigdis) x Agneta
Åsa	Sveriges Utsadesforening Svalöf	Dore x Vega
Birgitta	Sveriges Utsadesforening Svalöf	(Opal x Vega) x Maja
Birka	Weibullsholm Plant Breeding Institute	W 82-68 x W 17-68
Brage	Graminor Plant Breeding	Lavrans/NK91650
Brazil	KWS SAAT AG	Trebon x Cooper
Brio	Sveriges Utsadesforening Svalöf	Selection from 6-row barley from Skåne
Calisi	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Carlsberg	Carlsberg	Prentice x Maja
Caruso	Carlsberg	(Rupal X Terrax) X Grit
Chevallier Tystofte		
Cicero	Sejet Plant Breeding I/S	Chalice x SJ 933275

Accession name	Location / Breeder institute	Culton pedigree
Columbus	Sejet Plant Breeding I/S	Isabella x Publican
Culma	Limagrain	590907 x Hart
Danpro	Carlsberg	Proctor x Dana
Danuta	NordSaat	90014DH x (Salome x Maresi)
Denso Abed	The Abed Foundation	Rigel
Drost Pajbjerg	The Pajbjerg Foundation	Maja x Kenia
Edvin	Boreal Plant Breeding	Verner/Hja 85194
Elmeri	Boreal Plant Breeding	Thule/Verner
Etu	Agricultural Research Centre	Bonus M x Varde
Eva	Sveriges Utsadesforening Svalöf	Birgitta x Mari
Evergreen	Nordic Seed A/S Plant Breeding	Br6920b115*Quench
Fabel Sejet	Sejet Plant Breeding I/S	Newgrange x SJ 2256
Fairytale	Sejet Plant Breeding I/S	Colston x (Recept x Power)
Fero	Øtofte	Selection from Kenia
Fløya	(SFL)Norwegian Government Res.Station Holt	Selection from Ørnesbygg
Fræg	(SFL)Norwegian Government Res.Station Holt	Asplund x Maskin
Freja	Sveriges Utsadesforening Svalöf	Seger x Opal
Galant Carlberg	Carlsberg	Mutation in Triumph
Gaute	Graminor Plant Breeding	SvN82114/Vo13647-77
Gunnar	Svalöf AB	Kristina x ((Mari 6 x 57/510-44) x Å 61718)
Hafnia		Freja x Lenta
Hannchen	Sveriges Utsadesforening Svalöf	Line selection from Hanna
Harbinger	Boreal Plant Breeding	
Harriot	Nordsaat	(GS 1635 x Nordus) x Annabell
Helium	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Hydrogen	Nordic Seed A/S Plant Breeding	(Alis x Digger) x Derkado
Ida	Weibullsholm Plant Breeding Institute	Tellus x Arla M1
Iron	Nordic Seed A/S Plant Breeding	Marnie x LP 813.6.98
Jacinta	Nordic Seed A/S Plant Breeding	(Alexis x Meltan) x Canut
Jadar II	(SFL)Norwegian Government Res.Station Forus	Jadar x Asplund
Jotun	(SLF)Norwegian Government Res.Station Løken	Selection from the landrace Oppdalbygg
Juli Abed	The Abed Foundation	Collected material
Justus	Boreal Plant Breeding	
Karin	Svalöf AB	(Åsa x Edda II) x Varde
Karri	Hankkija Plant Breeding Station	Carlsberg x Rigel

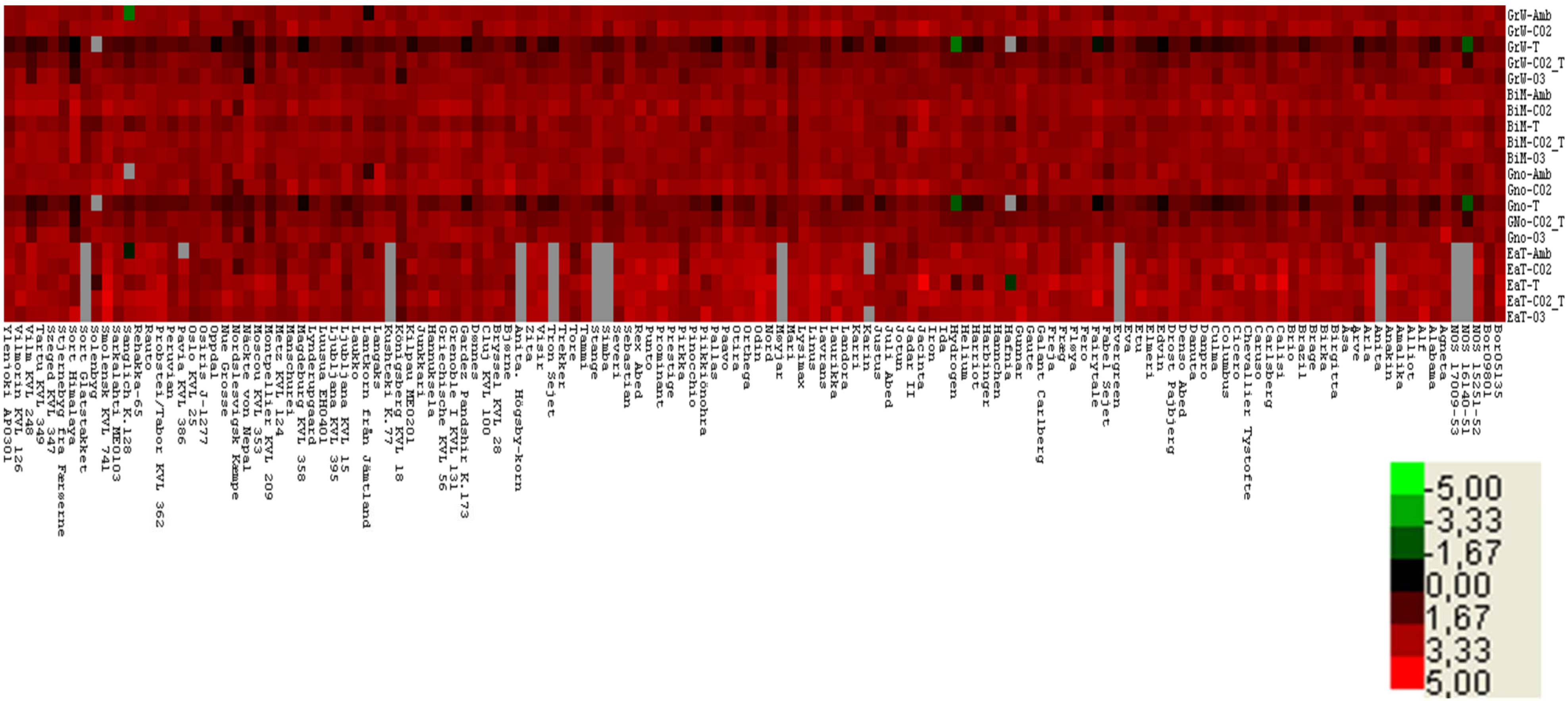
Accession name	Location / Breeder institute	Culton pedigree
Landora	Saatzucht Hadmersleben GmbH	Cross breeding (Hadm86508-91 x Hadm46544-88)
Laurikka	Nordic Seed A/S Plant Breeding	(Ceb 7931 x Pompadour) x (577223 x Golf)
Lavrans	Graminor Plant Breeding	Vera//Arve/H82009-1-3
Linus	Svalöf Weibull AB	WW 7749 x Ariel
Lysimax	Sejet Plant Breeding I/S	Ca 040223 x Carula
Mari	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Møyjar	(SFL)Norwegian Government Res.Station Møystad	Domen x Herta
Nord	Lantbrukets Forskningscentrals Växtförädlingsanstalt	Otra x Etu
Odin	Carlsberg	Sv 66433 x ALL.297
Orthega	KWS SAAT AG	Tucson x Quench
Otira	Sejet Plant Breeding I/S	Bartok x Sj 930331
Paavo		(Tammi x Gull) x O.A.C. 21
Pallas	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Piikkiönohra		Line from landrace from Piikkiö, Southwest Finlan
Pinocchio	Sejet Plant Breeding I/S	Quench x SJ Afrodite
Pirkka	Hankkija Plant Breeding Station	TA 04369 x TA 05864
Prestige	RAGT	AC 00/767/5 x Anakin
Prominant	Sejet Plant Breeding I/S	Caminant x Vintage
Punto	Sejet Plant Breeding I/S	Lamba x Meltan
Rex Abed	The Abed Foundation	Selection from old Danish 2-row
Sebastian	Sejet Plant Breeding I/S	Lux x Viskosa
Severi	Boreal Plant Breeding	
Simba	Sejet Plant Breeding I/S	Otira x Prolog
Stange	Norwegian Government Res.Station Møystad	Mari x Ingrid
Tammi	Hankkija Plant Breeding Station	Asplund x Olli
Tore	Norwegian Agrcultural University /(Graminor)	Lise x Clermont
Trekker	Boreal Plant Breeding	
Tron Sejet	Sejet Plant Breeding I/S	Impala x Nigrate
Visir	Sveriges Utsadesforening Svalöf	Pallas x Long Glumes
Zita	The Pajbjerg Foundation	PF 203/7748 x Vada
Anita Högsby-korn		
Bjørne		
Bryssel KVL 28		
Cluj KVL 100		
Dønnes		

Accession name	Location / Breeder institute	Culton pedigree
Gardez Pandshir K.173	Gardez Panshir	
Grenoble I KVL 131		
Griechische KVL 56		
Hannuksela		
Junkkari		
Kilpau ME0201	Kilpau, Oulainen	
Königsberg KVL 18		
Kushteki K.77	Kushteki	
Langaks	Faroe Islands	
Lantkorn från Jämtland		
Laukko		
Ljubljana KVL 15		
Ljubljana KVL 395		
Luusua EH0401	Luusua, Kemijärvi	
Lynderupgaard	Lyderupgaard	
Magdeburg KVL 358		
Manschurei		
Metz KVL 124		
Montpellier KVL 209		
Moscou KVL 353		
Näckte von Nepal		
Nordslesvigsk Kæmpe		
Nue Grosse		
Oppdal		
Osiris J-1277		
Oslo KVL 25		
Pavia KVL 386		
Peruvian		
Probstei/Tabor KVL 362		
Rauto	(SFL)Norwegian Government Res.Station Løken	
Rehakka-65		
Sanglich K.128	Sanglish	
Sarkalahti ME0103	Sarkalahti, Luumäki	
Smolensk KVL 741		
Solenbyg		

Accession name	Location / Breeder institute	Culton pedigree
Sort Glatstakket		
Sort Himalaya		
Stjernebyg fra Færøerne	Faroe Islands	
Szeged KVL 347		
Tartu KVL 349		
Vilm KVL 248		
Vilmorin KVL 126		
Ylenjoki AP0301	Jussila, Ylenjoki, Valkeakoski	
excluded, winter barley		
excluded, winter barley		Lady x 82101

APPENDIX 2

Heat-map of grain yield (GrW), number of grains (GNo), number of ears (EaT) and aboveground vegetative biomass (BiM) relative to ambient of 138 spring barley accessions grown in four climate treatments. The treatments were ambient (Amb; temperature: 19°C/12°, [CO_2]: 385 ppm), single factor treatments of elevated temperature (T; 24°C/17°C), [CO_2] (CO2; 700 ppb), and [O_3] (O3; 100-150 ppb) and a two-factor treatment of elevated temperature and [CO_2] in combination (CO2_T; 24°C/17°C, [CO_2]: 700 ppb). The GrW, GNo, EaT and BiM data sets were normalized by multiplication of all value by a scale factor S so that the sum of the squares of the values in each data set was 1.0 (a separate S was computed for each column).



APPENDIX 3

Stability indices over 5 treatments for average grain weight of one plant

accession name	culton type	Release year	m_i	S_i^2	W_i^2
Bor05135	BL		5.7	2.6	7.3
Bor09801	BL		6.5	2.8	13.9
NOS 15251-52	BL		5.9	4.7	4.8
NOS 16140-51	BL		4.1	8.2	6.0
NOS 17009-53	BL	expected 2015	6.2	3.8	2.0
Agneta	CV	1978	7.8	7.4	20.0
Alabama	CV	1999	5.6	6.1	2.4
Alf	CV	1978	5.7	6.1	4.7
Alliot	CV	1999	6.9	4.3	4.0
Amalika	CV	2012	6.9	4.9	5.6
Anakin	CV	2006	4.1	1.1	10.6
Anita	CV	1962	5.1	4.1	1.6
Arla	CV	1962	5.7	5.7	4.2
Arve	CV	1990	5.2	9.9	7.4
Åsa	CV	1949	6.2	1.2	8.8
Birgitta	CV	1963	5.4	1.1	3.4
Birka	CV	1981	6.5	4.7	1.6
Brage	CV	2010	6.7	6.5	4.7
Brazil	CV	2000	5.9	7.2	3.9
Brio	CV	1924	7.0	6.1	5.8
Calisi	CV	2013	5.8	1.9	4.3
Carlsberg	CV	1946	5.2	5.4	11.6
Caruso	CV	1991	6.8	3.8	0.9
Chevallier Tystofte	CV	1883	5.3	3.5	0.3
Cicero	CV	1999	6.3	8.0	4.5
Columbus	CV	2009	7.0	11.5	10.8
Culma	CV	1997	4.8	4.7	3.2
Danpro	CV	1969	6.8	11.6	9.3
Danuta	CV	2001	6.2	8.4	12.4
Denso Abed	CV	before 1965	5.3	2.9	1.8
Drost Pajbjerg	CV	1951	6.0	5.1	8.5
Edvin	CV	2008	5.1	10.0	8.2
Elmeri	CV	2009	4.7	5.3	1.8
Etu	CV	1970	5.1	4.8	0.8
Eva	CV	1973	5.7	4.1	4.4
Evergreen	CV	2010	7.0	3.8	2.6
Fabel Sejet	CV	2001	4.3	4.1	9.5
Fairytale	CV	2006	6.2	13.9	17.8
Fero	CV	1943	6.1	3.8	7.2
Fløya	CV	1939	5.8	3.2	1.3
Fræg	CV	1948	5.0	2.1	5.7
Freja	CV	1941	6.6	4.8	11.8
Galant Carlberg	CV	1985	4.6	1.2	3.4
Gaute	CV	2000	7.0	6.3	2.2
Gunnar	CV	1981	5.8	7.2	8.8
Hafnia	CV	1958	5.2	10.5	11.2
Hannchen	CV	1902	5.3	2.1	6.5
Harbinger	CV	2009	6.0	2.2	2.5
Harriot	CV	2001	4.9	3.9	4.0
Helium	CV	2001	4.9	6.2	3.3
Hydrogen	CV	2000	4.7	9.5	7.2

accession name	culton type	Release year	m_i	S_i^2	W_i^2
Ida	CV	1979	5.3	3.6	1.3
Iron	CV	2007	6.9	3.4	1.7
Jacinta	CV	1999	7.7	16.8	33.6
Jadar II	CV	1947	6.0	5.3	4.6
Jotun	CV	1930	6.2	5.7	2.4
Juli Abed	CV	1909	5.0	2.0	4.0
Justus	CV	2013	5.1	6.2	3.6
Karin	CV	1988	6.1	2.0	17.9
Karri	CV	1967	5.8	4.2	4.3
Landora	CV	2000	5.3	4.8	4.5
Laurikka	CV	2012	7.3	8.6	10.8
Lavrans	CV	1999	5.4	5.7	2.6
Linus	CV	1997	7.5	7.4	4.7
Lysimax	CV	1994	6.4	2.8	2.0
Mari	CV	1960	4.7	0.9	6.1
Møyjar	CV	1969	6.8	8.2	4.4
Nord	CV	1988	4.4	5.1	3.2
Odin	CV	1981	7.1	4.4	5.9
Orthega	CV	1997	6.8	11.1	11.0
Otira	CV	1997	6.2	4.1	0.3
Paavo	CV	1959	7.2	5.7	3.0
Pallas	CV	1970	5.2	9.0	5.7
Piikkiönohra	CV	1922	5.2	6.2	1.9
Pinocchio	CV	2011	5.6	5.7	1.5
Pirkka	CV	1952	5.0	2.2	5.4
Prestige	CV	2000	6.3	3.7	1.5
Prominant	CV	1999	6.5	6.4	5.9
Punto	CV	1995	4.7	1.0	7.9
Rex Abed	CV	1913	5.2	3.1	2.3
Sebastian	CV	2002	7.4	1.4	4.3
Severi	CV	2013	5.8	6.9	6.3
Simba	CV	2002	4.6	3.9	10.5
Stange	CV	1978	7.0	7.4	3.7
Tammi	CV	1937	5.5	3.2	1.8
Tore	CV	1986	5.2	0.9	4.8
Trekker	CV	2013	6.4	1.4	4.2
Tron Sejet	CV	1978	5.2	4.8	1.5
Visir	CV	1971	5.6	8.2	4.2
Zita	CV	1974	5.3	2.8	5.2
Anita Högsby-korn	LR		5.1	4.9	2.3
Bjørne	LR		6.6	6.8	3.6
Bryssel KVL 28	LR		5.9	3.5	3.2
Cluj KVL 100	LR		5.6	3.6	0.3
Dønnes	LR		4.9	2.9	16.5
Gardez Pandshir K.173	LR		5.8	8.2	6.3
Grenoble I KVL 131	LR		6.3	4.1	1.5
Griechische KVL 56	LR		7.1	5.1	4.4
Hannuksela	LR		6.6	5.8	3.0
Junkkari	LR		5.9	5.2	1.1
Kilpau ME0201	LR		5.6	6.2	10.4
Königsberg KVL 18	LR		4.2	2.7	11.9
Kushteki K.77	LR		6.5	5.3	5.8
Langaks	LR		5.3	4.6	5.2
Lantkorn från Jämtland	LR		4.6	6.2	25.9

accession name	**culton type**	**Release year**	m_i	S_i^2	W_i^2
Laukko	LR		4.6	2.9	2.7
Ljubljana KVL 15	LR		5.2	6.3	2.1
Ljubljana KVL 395	LR		6.2	3.3	2.5
Luusua EH0401	LR		5.4	1.9	4.5
Lynderupgaard	LR		6.1	5.1	1.6
Magdeburg KVL 358	LR		4.9	5.3	2.7
Manschurei	LR		7.0	12.0	13.5
Metz KVL 124	LR		6.3	2.0	8.5
Montpellier KVL 209	LR		3.8	1.1	3.8
Moscou KVL 353	LR		6.1	1.7	4.6
Näckte von Nepal	LR		3.0	4.3	13.8
Nordslesvigsk Kæmpe	LR		4.1	4.9	25.5
Nue Grosse	LR		4.7	2.0	1.0
Oppdal	LR		5.4	6.4	5.9
Osiris J-1277	LR		6.0	2.8	4.7
Oslo KVL 25	LR		4.7	0.6	12.3
Pavia KVL 386	LR		6.2	8.2	8.0
Peruvian	LR		6.5	3.3	1.7
Probstei/Tabor KVL 362	LR		5.5	1.7	3.0
Rauto	LR		5.1	3.5	1.8
Rehakka-65	LR		5.6	3.1	0.6
Sanglich K.128	LR		3.7	9.2	29.4
Sarkalahti ME0103	LR		6.4	9.9	7.5
Smolensk KVL 741	LR		5.5	5.8	4.8
Solenbyg	LR		5.9	11.9	13.4
Sort Glatstakket	LR		5.9	1.3	4.8
Sort Himalaya	LR		3.9	10.9	12.7
Stjernebyg fra Færøerne	LR		4.5	2.6	7.4
Szeged KVL 347	LR		6.8	4.3	4.8
Tartu KVL 349	LR		6.2	8.0	6.2
Vilm KVL 248	LR		4.8	11.9	15.4
Vilmorin KVL 126	LR		5.4	4.3	13.0
Ylenjoki AP0301	LR		5.0	3.4	2.4

BL_ breeder-lines, CV: cultivar, LR: landrace, m_i: mean yield across treatments, S_i^2: environmental variance, W_i^2: Wricke's ecovalence

Manuscript 2

Genome-wide association study of production and stability traits in barley cultivated under future climate scenarios

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Keywords *Climate change, genetic structure, genome-wide association, Hordeum vulgare, multi-factor, SNP*

Abstract

To assist the breeding of spring barley for climate change conditions 127 accessions – from landraces to breeder-lines – were grown under future climate scenarios and genome-wide association was applied. In the future climate scenario, elevated temperature (+5 °C) and carbon dioxide ([CO_2]; 700 ppm) were combined according to projections in the IPCC SRES A1FI scenario of IPCC. Also single-factor treatments of temperature, [CO_2] (700 ppb) and ozone ([O_3]; 100-150 ppb) were applied. An ambient treatment mimicked south Scandinavian summer of 19/12 °C (day/night). Phenotyping included grain yield, number of grains, number of ears and vegetative biomass, harvest index and also stability of the production parameters over all applied treatments. Genotyping comprised 7864 SNP markers (Illumina array). Genome-wide association was applied using a compressed mixed linear model with the GAPIT package, and conservative validation of markers was performed to avoid false positives. A total of 60 marker-trait associations (log_{10}(p) 2.97-5.58) from 25 LD blocks were identified, and hereof seven associated with grain yield. Two LD blocks on chromosome 4H and 7H associated with grain yield under elevated [CO_2]. Three marker-trait associations were established from the two-factor treatment, but only one of these was also present in one of the single-factor treatments. Markers associated with stability over treatments were also found on 1H, 4H, 5H, 6H and 7H. This paper reports markers and chromosome regions to be targeted in future breeding for climate resilient cultivars and discusses the function of genes co-localised with associated markers.

Electronic supplementary material

The 'online resources' are available in print in the dissertation and digitally on the attached CD-rom.

Abbreviations

AllM	data set of all markers
BM	aboveground vegetative biomass
EG	number of ears with grains
ET	number of ears
GN	number of grains
GWAS	genome-wide association
GY	grain yield
HI	harvest index

LD	linkage disequilibrium
MwP	data set of the markers of AllM with position
QTL	quantitative trait loci
SNP	single nucleotide polymorphism

Introduction

Rapid changes in growth environments induced by altered climatic conditions urge the need to develop climate resilient crop cultivars through breeding. Traditionally, introduction of genes into elite germplasm has increased resistance to pest and pathogens, but with climate change also abiotic stress demands focus in crop development. Barley (*Hordeum vulgare* L.) is an important crop plant widely used as feed for livestock and in food and beverage products for human consumption. Hence, barley is an economically important crop (Newton et al. 2011).

Studies have reported high phenotypic plasticity in wild barley, which can be the explanation for its large geographical distribution and wide adaptation to diverse environmental conditions (Nevo et al. 2012). As a diploid, inbreeding, temperate crop, barley has traditionally been considered a model for plant genetic research. Large collections of germplasm containing geographically diverse elite varieties, landraces and wild accessions are readily available and possibly contain alleles that could ameliorate the effect of climate change (The International Barley Genome Sequencing Consortium 2012).

Marker-assisted selection, where markers for desired agricultural traits are applied to verify loci related to a phenotype, is a method to accelerate plant breeding. However, reliable markers are needed. In recent years, the cost of genotyping has decreased considerably (Wetterstrand 2014), and accelerated the identification of markers associated with agricultural traits encoded by quantitative trait loci (QTL) in segregating crosses, and genome-wide association (GWA) in diverse set of accessions. The emergence of high throughput SNP marker genotyping platforms enabled the implementation of GWA in barley (Close et al. 2009; Waugh et al. 2009). The advantage of GWA to traditional mapping is that alleles present within the diverse set of accessions can be identified, and not only alleles present in the parents of segregating crosses (Zhu et al. 2008). Because GWA analyse the linkage disequilibrium (LD) between marker-loci, the genetic structure (relationships) within the set of accessions must be accounted for. Also, the frequency of an allele must reach a sufficient level (minor allele frequency) in order to avoid an inflated rate of false positives. Hence, GWA are limited in their ability to find rare alleles (Tabangin et al. 2009).

LD is found to be extensive in barley (Caldwell et al. 2006; Comadran et al. 2011), and therefore barley is appropriate for identification of marker-trait associations by GWA. Numerous studies have reported QTLs and marker-trait associations for agricultural traits in barley (e.g. Kraakman et al. 2004; Schweizer and Stein 2011; Varshney et al. 2012; Tondelli et al. 2013), studies that have also led to better understanding of plant tolerance to e.g. salt and aluminium (Cai et al. 2013; Long et al. 2013). Exploring QTLs and marker-trait associations under climate change conditions could improve the understanding of genes and processes operating under such conditions. However, the phenotype effect of climate change has not yet been considered in a GWA study.

Lobell et al. (2011) found that climate impacts and prevailing temperature increases in the period from 1980 to 2008 have already decreased the global wheat yield by 5.5 %. According to IPCC (Intergovernmental Panel of Climate Change; 2013) the concentration of atmospheric carbon dioxide ([CO_2]) can, in the worst-case scenario (RCP8.5) reach around 1,000 ppm (mean value) at the end of the 21st century from approximately 400 ppm of today. In the same period the concentration of atmospheric ozone ([O_3]), being 32-62 ppb today (Ellermann et al. 2013), is expected to increase 8 ppb averaged per year. The elevated greenhouse gasses are in the most unpleasant situation foreseen to increase temperature 5 °C (IPCC 2013). Further, the temperature of the hottest seasons experienced in temperate regions today is expected to be the norm by the end of the 21st century (Battisti and Naylor 2009).

Even though plants have a long history of adaptation, climate change might be progressing with a pace that outcompetes the natural adaptation (Rosenzweig and Parry 1994; Svenning and Sandel 2013). In recent years, barley production has stagnated in the high producing countries of Europe (FAOSTAT 2014). Hence, to maintain and preferable increase crop production to meet the need of higher living standards and population growth (UN 2012; IPCC 2014), the cultivars of the future should be designed to exploit the elevated [CO_2] and possess stability towards extreme climate shifts during the growing season. Here we applied 7864 SNP markers to a set of spring barley accessions of landraces, old and new cultivars as well as breeder-lines in the search for associations with phenotyped production traits e.g. grain yield and it components, biomass and stability of performance under elevated levels of temperature (+5 °C), [CO_2] (700 ppm) as single-factors and in combination and elevated [O_3] (100-150 ppb) as a single-factor.

Material and methods

Germplasm

A total of 127 predominantly Nordic but also European spring barley accessions of both 2- and 6-row types were included in the analysis. They were 38 landraces, 31 old cultivars (1883-1974), 53 modern cultivars (1975-2013) and 5 breeder-lines. The separation into new and old cultivars was based on the introduction of exotic gene pools as disease resistance in the period after 1975 (Backes et al. 2003). The type of accessions, row-type and place of origin are listed in Table 1, and further details of the material are shown in Online Resources 1.

Phenotyping

The experimental set up and results are described in more detail in Ingvordsen et al. (2014). The accessions were grown to maturity under five different climatic conditions based on the IPCC prediction for levels of [CO_2], [O_3] and temperature by the year 2100 (SRES A1FI, IPCC 2007) in the RERAF phytotron (http://www.eco.kt.dtu.dk/Research/Research_Facilitites/RERAF). The five treatments were (1) ambient (amb) of 19/12 °C (day/night), [CO_2] at 385 ppm; (2) elevated [CO_2] (CO_2) at 700 ppm; (3) elevated temperature (tmp) 24/17 °C; (4) elevated [O_3] (O_3) 100-150 ppb. Ozone was only added in the elevated [O_3] treatment. In treatment (5) elevated temperature 24/17 °C and [CO_2] at 700 ppm were combined. Humidity and amount of added water and fertilizer were identical between all treatments. Eight plants of each accession were grown in large pots of 23 cm × 23 cm and 11 L (plant density was 151 plant/m^2), and all applied production parameters were averaged from eight plants.

After harvest the following production parameters were measured for each accession and treatment: grain yield (GY, g $plant^{-1}$), number of grains (GN, no. $plant^{-1}$), aboveground vegetative biomass (BM, g $plant^{-1}$), total number of ears (ET, no. $plant^{-1}$) and numbers of ears with grains (EG, no. $plant^{-1}$). The harvest index (HI, %) was calculated as grain yield relative to aboveground vegetative biomass, and Δ (delta) values were calculated as the production parameter of a given accession in a climate treatment relative to the ambient treatment. The static stability environmental variance (S^2; Roemer, 1917) and the dynamic stability Wricke's ecovalence (W^2; Wricke, 1962) were determined for all production parameters over the five treatments. S^2 is defined as:

$$S^2_i = \sum (R_{ij} - m_i)^2 / (e - 1) \qquad [1]$$

where R_{ij} is the observed yield of the accession i in the treatment j, m_i is mean yield of the accession across treatments, and e the number of environments. W^2 is defined as:

$$W^2_i = \sum (R_{ij} - m_i - m_j + m)^2 \qquad [2]$$

where R_{ij} is the observed yield of the accession i in the treatment j, m_i is mean yield of the accession across treatments, m_j is mean yield across treatment j of all accession and m is the grand mean, average of all m_i.

Genotyping

Genomic DNA was extracted using the CTAB procedure (Cetyl Trimetheyl Ammonium Bromide; Rogers and Bendich 1985). Plant material was collected at seedling stage and freeze dried. For cultivars and breeder-lines DNA was extracted from one individual. However, for landraces two to six individuals were included and treated as separate genotypes. By this, in total 192 genotypes were analysed from the 127 accessions. A total of 7864 SNP (single nucleotide polymorphism) markers were provided by the Illumina array and analysis performed by TraitGenetics (TraitGenetics GmbH, Gatersleben, http://www.traitgenetics.com/en/).

Genetic structure and association analysis

Genetic structure between accessions was analysed using a distance matrix based on “Simple Matching” and a subsequent principal coordinate analysis (PCoA). This was computed using the macro ‘Diversity’ in Microsoft Excel (2010) (programmed by G. Backes, co-author). Also a Bayesian Cluster Analysis using the software “STRUCTURE” (Pritchard et al. 2000) was applied on the data set. For the choice of the optimal number of groups (K), the method of Evanno et al. (2005) was applied. Linkage disequilibrium for a random sample of SNP marker pairs with a distance smaller than 50 cM (10% of all linked pairs) was calculated and visualised in Microsoft Excel (2010) by the macro ‘Assoc’ (programmed by G. Backes, co-author).

GWA was performed using a compressed mixed linear model taking genetic structure within the set of accessions and genetic relatedness into account (Zhang et al. 2010) by use of the macro ‘Assoc.’ The macro ‘Assoc.’ calls the R-package Genomic Association and Prediction Integrated Tool, GAPIT, (Lipka et al. 2012) by use of R version 2.15.3 (R core Team 2013).

GAPIT apply efficient mixed-model association (EMMA) for statistical testing of association mapping and correcting for possible structure and genetic relatedness (Kang et al. 2008). Optimization of the model for the association analysis was performed for each phenotype with two partly overlapping datasets. One data set included all SNP markers (AllM), whereas the second dataset was the subset of AllM including only SNP markers with a known genome position (MwP). Association analysis was performed on each of the two datasets with the optimized model including genetic structure and a naive model not included genetic structure. Associations were only accepted if they had $\log_{10}(p) > 2.95$ for both dataset in both analyses.

Linkage disequilibrium and bioinformatics of associated markers

Linkage disequilibrium (LD) was determined for associated markers positioned on the same chromosomes by TASSEL version 4.0 (Bradbury et al. 2007). When LD was found, the respective markers were treated as a LD block. The position of the LD block was, besides determined by cM, determined as bin according to the BinMap 2005 with its unique segregation patterns separating the bins by single recombinant events. In practice GrainGenes 2.0 (http://wheat.pw.usda.gov) was utilized and the barley maps OPA123-2008, OPA 2011, OWB, Stein 2006 and/or OWB OPA2008 when necessary. Known genes that co-localised with SNP markers were determined through marker position from HarvEst Barley version 2.02, available at http://harvest.ucr.edu/ (Alpert et al. 2011).

Results

Effects of applied treatments

The treatment effects on the set of the production parameters are described more detailed in Ingvordsen et al. (2014). In general the production parameters were found to be strongly affected by the climatic conditions (Table 2), and differences between cultivar responses were prominent as also reported in (Ingvordsen et al. 2014). Elevated temperature decreased grain yield (56 %), number of grains and also aboveground vegetative biomass and HI. Elevated [CO_2] increased all production parameters apart from HI. In the two-factor treatment grain yield decreased (30 %) together with number of grains, whereas number of grain-bearing ears and total number of ears increased; the aboveground vegetative biomass was overall not influenced. Elevated [O_3] resulted in reduced grain yield (16 %), but had a positive effect on number of ears produced (Table 2). The stability measures calculated over the applied treatments varied from 0.58 to 16.79 for the environmental variance S^2 and 0.27 to 33.60 for the dynamic stability W^2 as described in Ingvordsen et al. (2014).

Marker analysis

Out of the 197 tested barley genotypes, 167 representing 127 accessions (2-6 individuals per landrace) held information from more than 60 % of the SNP markers and were included in the GWA analysis. Polymorphism was obtained for 84% (6624) of the SNP markers (the AllM dataset) and 3953 of these SNP markers had an assigned genomic position (the MwP dataset). Heterogenic genotypes were excluded. All phenotypic traits, except for ΔET under elevated [O_3] passed the optimization step and were included in the GWA. The LD decay of all SNP markers was found to decrease considerable after 40 cM and sufficient to perform GWA (Fig 1).

Genetic structure

Three groups were recognized within the 127 accessions by the principal coordinate analysis (PCoA) (Fig 2) and the Bayesian clustering (Online Resources 2). The groups corresponded reasonably well with row type and time of release. One group comprised primarily by 6-rowed accessions of different age (landraces, old- and modern cultivars) (Fig 2, III), whereas the 2-rowed accessions were divided into two groups by time of release. One group of 2-rowed accessions included modern cultivars (Fig 2, I) and the other old cultivars and landraces (Fig 2, II). No grouping could be identified by country of origin.

Marker associations and LD

Sixty SNP-marker-phenotype trait associations with $\log_{10}(p)$ values from 2.97 to 5.58 were found linked to 44 different SNP markers (Table 3, Online Resources 3). The traits that associated most frequently were GY, ΔGY, ΔGN, EG and HI and most marker-trait associations were found in the treatments with elevated [CO_2] and temperature as single-factors. Few markers were found associated with S^2 and phenotypes in the two-factor treatment. No associations were found for ΔEG under either of the climate scenarios.

Analysis of LD between the 44 markers revealed 25 independent LD blocks (Table 3, Online Resources 4) and 9 of these groups included more than 1 SNP marker. In Table 3 the SNP marker with the highest log_{10}(p)-value within a LD block is reported. All marker-trait associations are reported in Online Resources 3. Several markers were associated with more than one phenotype, i.e. some of the LD blocks with one SNP marker were associated with more than one phenotypic trait (Table 3, Online Resources 3. 4). Generally, a LD block was associated with similar phenotypic traits across climatic conditions, e.g. LD block 3 for GY (Table 3). LD block 6 and 19 was associated with different phenotypic traits in different climatic treatments, i.e. ΔGY under elevated temperature together with ΔET in the two-factor treatment (LD block 6, Table 3) and dynamic stability W^2 for HI together with EG under elevated temperature (LD block 19). Only LD block 6 included marker-trait associations from a single-factor as well as the two-factor treatment. Marker-trait associations related to elevated [CO_2] were only identified in LD blocks with more than one marker, while marker-trait associations related to elevated [O_3] were also found in LD blocks with one marker. LD block 4, 5 and 6 includes eight SNP markers that all associate with GY, ΔGY, BM, ΔBM or ET under elevated temperature (Table 3). The marker in LD block 8 was associated with three phenotypes; ΔGY, ΔGN and ΔHI under elevated temperature. Associations for GY, ΔGY and ΔGN under elevated [CO_2] were also found with six markers in LD block 10 and 22. For the two-factor treatment three marker-trait associations were identified in LD block 6, 14, and 21 for ΔET, HI and ΔGN respectively.

Markers associating for climate-stability of accessions

Nine markers representing eight LD blocks were found to associate with the climate-stability of accesions from measured genotype traits across the five climatic treatments, with 3 SNP markers associated with S^2 and 6 markers with W^2. Two of the markers for S^2 of HI and GY were both found in LD block 25. The marker that associated with W^2 of GY was found in LD block 22 together with marker-trait associations for ΔGN, ΔGY and GY under elevated [CO_2].

Discussion

We found SNP markers to associate with grain yield, number of ears and grains, vegetative biomass and HI as well as stability of these traits under future levels of elevated [CO_2], [O_3] and temperature in both single- and a two-factor treatments under controlled conditions. To our knowledge this is the first study to report on markers associated with production traits scored under complex climate change conditions.

Genetic structure among accessions in the test-set

The observed genotypic separation of the 127 barley accessions according to time of release as well as row-type (2- and 6-rowed) has also been reported in earlier studies with European and Nordic spring barley accessions (Backes et al. 2003; Brantestam et al. 2004; Brantestam et al. 2007). As in the present study, no clear grouping according to 'country of origin' were identified by Brantestam et al. (2007). Even though the agro-environmental conditions for cultivation of spring barley differ widely in the Northern European region, local adaptation or breeding for regional conditions could be present, obscuring the national origin. However, the lack of grouping according to 'country of origin' within the Nordic countries Denmark, Finland, Norway and Sweden could also suggest that the cultivars might possess a wide adaptation to Nordic conditions or that they represent common germplasm independent of national breeding programs.

Conservative validation of marker-trait associations

The outcome from GWA studies is the results of the applied statistical model and dependent on the set of accessions studied. Additionally, type of molecular markers as well as method and phenotypes scored will influence the output. Several studies have debated the statistical challenges and the inputs (Jannink 2007; Stich et al. 2008; Matthies et al. 2011a). In the present study both a naive model - not including genetic structure - and a linear mixed model including genetic structure by kinship (EMMA) were applied on two data sets, where one was a subset of the other. In the conservative approach applied here, only associations that were significant ($\log_{10}(p) > 2.95$) in all four analyses were accepted. This conservative approach was applied in order to avoid false-positives; however, a higher rate of possible marker-trait associations was consequently rejected.

The conservative procedure of validation was decided on to achieve marker-trait associations with known chromosome localization solid enough to be exploited directly in marker assisted selection.

Markers for breeding of cultivars for the future climate

Since 1975 [CO_2] have risen from 330 ppm to 400 ppm of today (IPCC 2013). However, no studies have so far identified a response to the experienced change in [CO_2] through grain yield of modern cultivars, despite that modern cultivars have been developed along with the gradually increasing [CO_2] (Manderscheid and Weigel 1997; Ziska et al. 2004; Franzaring et al. 2013; Ingvordsen et al. 2014). The markers in the present study, which associated with grain yield and grain number under elevated [CO_2] identified in LD block 10 and 22, are possible targets, when aiming to improve responsiveness of the grain yield to elevated [CO_2]. Further, a putative sucrose synthase, a key enzyme in the sucrose metabolism forming carbohydrates (Barrero-Sicilia et al. 2011), is co-localizing with one of the markers in LD block 22 (BOPA2_12_30880; Alpert et al. 2011). Marker associations with grain yield have previously been reported to localize here (Matthies et al. 2011b).

The identification of markers associated with either grain yield or vegetative aboveground biomass under elevated temperature on 2H (LD block 3 and 4) calls for further investigations on functional genes in that area. The presence of loci beneficial for grain yield production in this chromosome region has previously been reported by Varshney et al. (2012) and Hayes et al. (1993). Interestingly, the pseudo-response regulator gene *Ppd-H1*, found to provide adaptation to different environments through photoperiod responses, is positioned in this region on 2H (Turner et al. 2005; Wang et al. 2010). Within the QTL reported by Wang et al. (2010) the strongest candidate gene for *Ppd-H1* was found located at 2H.3, where we have identified four markers associated with grain yield or grain yield relative to ambient under elevated temperature (Table 3, LD block 3). However, the bins span large genomic areas and connection remains therefore speculative.

In LD block 5, which spanned 40.7 cM, five markers associated either with ears with grains or total number of ears under all applied treatments except for the two-factor treatment. The gene product co-localizing with the marker positioned at 66.3 cM (first marker) show homology to a *PsbP* family protein, which plays a role in the oxygen evolving complex of photosystem II that is essential for normal photosynthetic activity (Roose et al. 2007; Alpert et al. 2011). The marker at 66.9 cM, also within LD block 5, that associated with total number of ears under elevated temperature, but also under elevated [CO_2], co-localize with a putative peroxidase 18-like protein.

Several peroxidases have been found involved in abiotic and biotic stress responses and play a role in roots under drought. In the present study water was applied in equal amount over treatments, and therefore possible drought effects will have been most expressed in the treatment of elevated temperature due to vapour pressure deficit. However, the type of the co-localizing peroxidase was not researched. The marker that associated with total number of ears under elevated temperature, located in the LD block 5, could be a useful breeding tool, since elevated temperature previously have been found to decrease ear production (Köszegi et al. 2005) – assuming, however, that the ears will also hold grains.

On 5H all LD blocks included only one marker. Three of the LD blocks on 5H were found to associate with HI under elevated temperature or elevated temperature in combination with elevated [CO_2] (Table 3). Two of the markers were located within genes for a protein kinase family and a casein kinase II subunit alpha 2 (Alpert et al. 2011). Kinases are active in numerous plant processes e.g. stress-responsive pathways, conserved regulation and in adaptive processes (Mulekar et al. 2012; Lehti-Shiu and Shiu 2012). It is possible that the diurnally elevated temperature in RERAF has stressed the plants, which could explain the co-localization with kinases.

Even though we approached genotypic differences in climate-stability of growth and yield performance under different conditions by having two stability parameters, the complexity of these growth processes remained highly speculative. An accession with a low value of S^2 is interpreted as environmentally stable, which is likely preferable under the expected future climate. The marker associated with S^2 of grain yield, found in LD block 25 together with another marker associated with S^2 of HI, co-localize with ubiquitin-conjugating enzyme 18 (Alpert et al. 2011). A co-localization with ubiqutin, involved in protein degradation, indicates the involvement of protein degradation in stability to climatic factors. However, the complexity of the processes that govern yield will make an interpretation highly proposed; this complexity might also be the reason for the scarcity of published results for QTLs for stability in barley (Kraakman et al. 2004; Lacaze et al. 2009). However, not least due to the threat of climate change, this area of research is developing (reviewed by Korte and Farlow 2013) valorising the potential for markers-trait associations for stability.

Marker-trait associations in the two-factor treatment differ from those in the single-factor components

In the two-factor treatment three marker-trait associations were identified, explicitly for the phenotypes number of grains relative to ambient, total number of ears relative to ambient and HI. Only three markers were found associated in the two-factor treatment and 31 under the corresponding single-factor treatments. It is likely that the responses in the combined treatment involve the interplay of more genes, and therefore associations cannot easily be detected by GWA. One could assume that a marker or LD block associated with grain yield under either elevated temperature or [CO_2] would also be associated with grain yield, when both of these single-factors were elevated simultaneously. Interestingly, only in one LD block (Table 3, 6) associations were found both for the two-factor treatment and one of the component single-factor treatments (temperature) associating with total number of ears relative to ambient (two-factor) and grain yield relative to ambient (temperature). The absent overlap of marker-traits from single-factor and double factor treatments may be attributable to the opposite effects of elevated temperature and [CO_2] on grain yield (Table 3). Nevertheless, the apparent absence of similarity in genetic regulation of effects in the combined and the single-factor treatments emphasizes the need for multi-factor studies to develop markers for the phenotypes favourable under multifaceted future climate conditions. Despite the need for cultivars, that can secure the future food production, there are to the authors' knowledge no GWA studies on multi-factor treatments mimicking the future climate performed on any crop plant. Even in *Arabidopsis*, associations have only been found under single-factor treatments of elevated temperature and [CO_2] (Assmann 2013).

Preferably, association studies should be performed under field conditions with elevated climatic factors. However, multifactor field studies including temperature are challenging in setup. Despite several attempts and developments of technologies, it is difficult to increase temperature more than 1-2°C under field conditions without several redundant experimental effects (Kimball et al. 2007; Bruhn et al. 2013). For GWA analyses an appropriate – and large - quantity of accessions must be included, and that defines the size of the facility used to manipulate the climate, and further exclude several enclosure facilities.

In conclusion the use of GWA, bridged the phenotype as expressed under future climate conditions with the genotype established by numerous SNP markers. Our results have revealed potential genome sites to be explored in breeding of cultivars; cultivars that can meet the needs under changed climatic conditions. Further, the results can contribute to the understanding of the genetic mechanisms behind cultivar's improved resilience against climatic constraints. SNP-candidates to be introduced as markers in marker-assisted selection are reported and might lead to breeding of cultivars resilient to the future climate.

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Table 1 Barley accessions sorted according to country of origin, accession type and sub type.

	Landrace		Old cultivar		Modern cultivar		Breeder's line	
Country	2 row	6 row	2 row	6 row	2 row	6 row	2 row	6 row
Belgium		1			1			
Croatia	1	1						
Czech Republic	1							
Denmark	1	2	9	1	25		3	
Estonia	1							
Farao Islands		2						
Finland[a]	2	5	2	4	2	5	1	1
France	1	2			2			
Germany	2				4			
Greece		1						
Hungary	1							
Italy	1							
Norway		4	1	5	1	5		
Romania		1						
Sweden	1		7	2	6	2		
Unknown		5						
TOTAL	12	24	19	12	41	12	4	1

[a]2 accessions segregated both in 2 and 6 rowed.

Table 2 Effect of climatic treatments on production parameters in relation to ambient (%) and the variance for the 127 accessions (Ingvordsen et al. 2014). Production given on a "per plant basis".

	ambient	+ CO_2		+ tmp		+ CO_2 & tmp		+ O_3	
	var	%	var	%	var	%	var	%	var
Grain yield (g)	1.31	14.5***	3.77	-55.8***	1.43	-29.6***	1.32	-15.8***	1.69
Number of grains (#)	844	13.8***	1419	-47.5***	677	-23.7***	634	-5.8	916
Number of ears with grain (#)	1.89	9.3*	2.92	-4.3	4.63	13.1***	3.10	6.2	3.30
Number of ears total (#)	2.13	9.5*	3.77	18.7***	7.66	18.8***	3.70	9.5*	3.69
Aboveground vegetative biomass (g)	1.72	16.5***	4.33	-12.1***	2.94	3.3	2.71	-0.9	1.54
Harvest index (%)	0.03	-1.1	0.04	-47.8***	0.05	-31.2***	0.03	-15.2***	0.02

Table 3 Marker-trait associations identified from four climate treatment and an ambient treatment. Var. expl. (%): part of the genotypic variance explained by the marker, effect: trait-difference between the allelic groups and SD the belonging standard deviation.

LD block	Most significant marker	Chrom	Pos (cM)	$log_{10}(p)$	Var. expl.	Effect	SD effect	Phenotype	Span of LD block (cM)	# of markers	Additional phenotypes
1	i_SCRI_RS_232577	1H.1	5.0	4.32	8.0	2.687	8.400	W^2 for EG	5.0-5.0	3	ET, EG, amb
2	i_12_31276	1H.3	32.2	3.12	6.3	1.053	2.936	S^2 for GY		1	
3	i_SCRI_RS_143250	2H.3	23.0	3.87	8.8	0.438	1.197	GY, tmp	23.0-23.8	4	ΔGY, tmp
4	i_12_30657	2H.3-4	39.7	3.97	6.1	0.804	1.907	BM, tmp		1	ΔBM, tmp
5	i_SCRI_RS_6727	2H.6	66.9	5.58	8.0	1.332	2.864	ET, tmp	66.3-107.0	5	EG, amb; EG, ET, CO_2; EG, O_3
6	i_SCRI_RS_129178	2H.8-9	114.1	3.27	8.1	0.706	1.817	ΔGY, tmp	113.5-114.1	2	ΔET, ×2
7	i_SCRI_RS_167825	3H.7	100.3	3.61	7.7	0.626	1.387	GY, O_3		1	
8	i_SCRI_RS_144313	3H.9-10	135.5	4.34	9.5	23.213	40.098	ΔGN, tmp		1	HI, GY, tmp
9	i_12_30992	4H.3-4	43.3	3.96	9.4	0.611	1.402	ΔBM, O_3	43.3-43.8	3	BM, GY, amb
10	i_11_11405	4H.4	49.9	4.87	10.3	12.941	34.971	ΔGN, CO_2	49.7-83.6	4	ΔGY, CO_2; ΔGY, O_3
11	i_SCRI_RS_192689	4H.7	104	3.18	6.2	0.030	0.081	W^2 for HI		1	
12	i_11_20553	5H.1	0.1	4.01	5.5	1.088	1.819	EG, O_3		1	
13	i_11_11048	5H.2	23.6	3.20	7.2	0.347	1.339	ΔBM, O_3		1	
14	i_SCRI_RS_144841	5H.3	50.0	4.05	9.4	0.088	0.177	HI, ×2		1	
15	i_SCRI_RS_162696	5H.7	113.9	3.44	7.1	0.092	0.226	HI, tmp		1	
16	i_SCRI_RS_166857	5H.8-9	128.1	3.11	6.3	0.072	0.222	HI, tmp		1	
17	i_11_10536	5H.9	144.5	2.98	5.1	3.898	8.678	W^2 for EG		1	
18	i_11_20996	6H.6	88.6	3.60	7.1	3.010	6.834	W^2 for BM		1	
19	i_SCRI_RS_8034	6H.7	100.4	3.94	8.1	0.053	0.091	W^2 for HI	100.4-100.8	2	EG, tmp
20	i_SCRI_RS_93773	7H.1	0.3	3.41	7.1	10.653	30.216	GN, O_3		1	
21	i_SCRI_RS_213333	7H.2	21.4	2.97	5.6	11.972	29.538	ΔGN, ×2		1	
22	i_12_30880	7H.5	54.4	5.27	11.4	19.371	37.005	ΔGN, CO_2	54.4-55.0	3	GY, ΔGY, CO_2; W^2 for GY

23	i_SCRI_RS_204256	7H.7	91.2	3.70		7.0	0.968	1.769	BM, tmp		1	
24	i_SCRI_RS_107367	7H.7	108.1	3.55		7.7	0.065	0.168	HI, O_3		1	
25	i_SCRI_RS_140746	7H.8	120.4	3.66		7.7	0.870	2.910	S^2 for GY	120.0-120.4	2	HI, tmp; S^2 for HI

amb: ambient, BM: aboveground vegetative biomass, Chrom: chromosome, ET: total number of ears, EG: numbers of ears with grains, GN: number of grains, GY: grain yield, Pos: position, tmp: temperature, S^2: environmental variance, W^2: Wrickes' ecovalence, ×2: two-factor, Δ: in relation to the ambient treatment

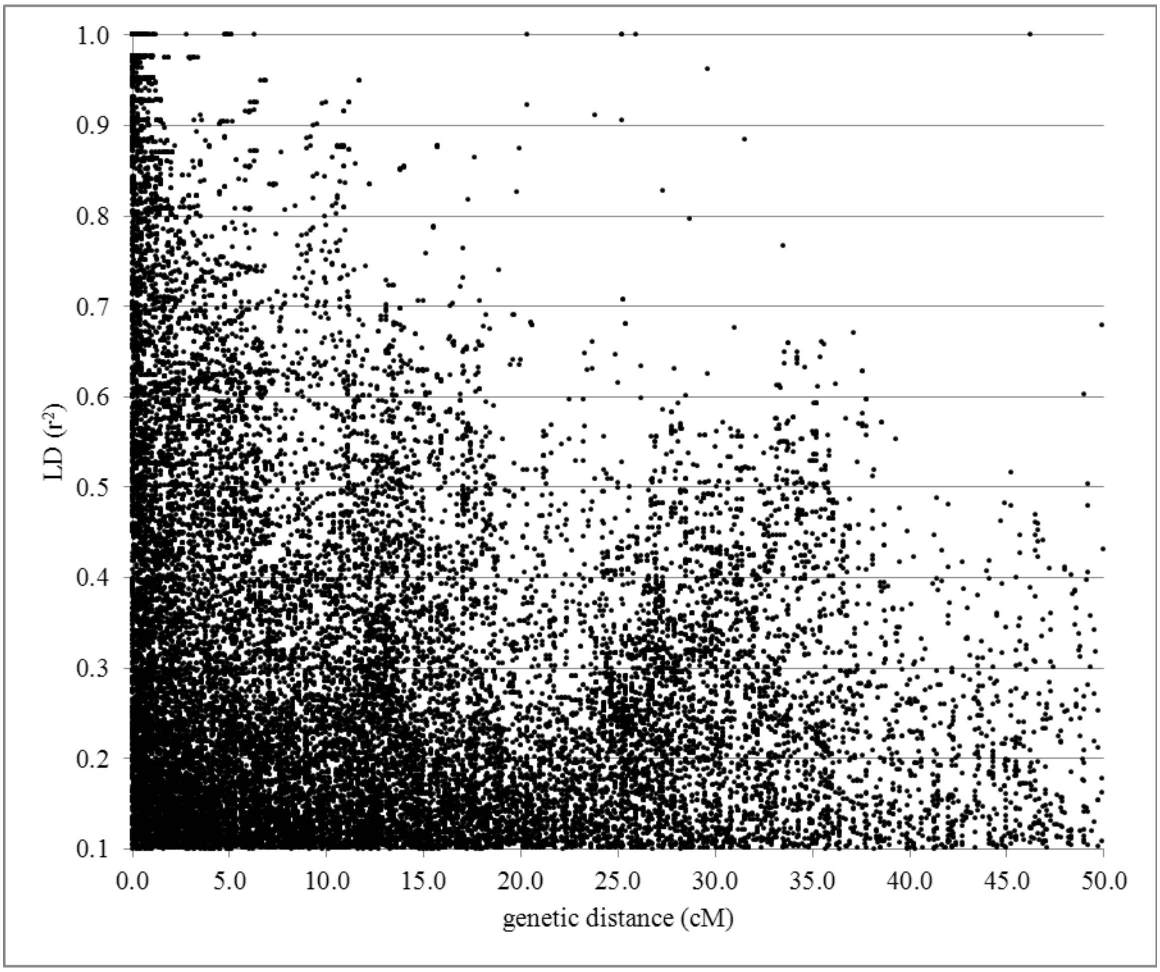


Fig 1 Decay of linkage disequilibrium (LD) from 0-50 cM of the 7864 SNP markers.

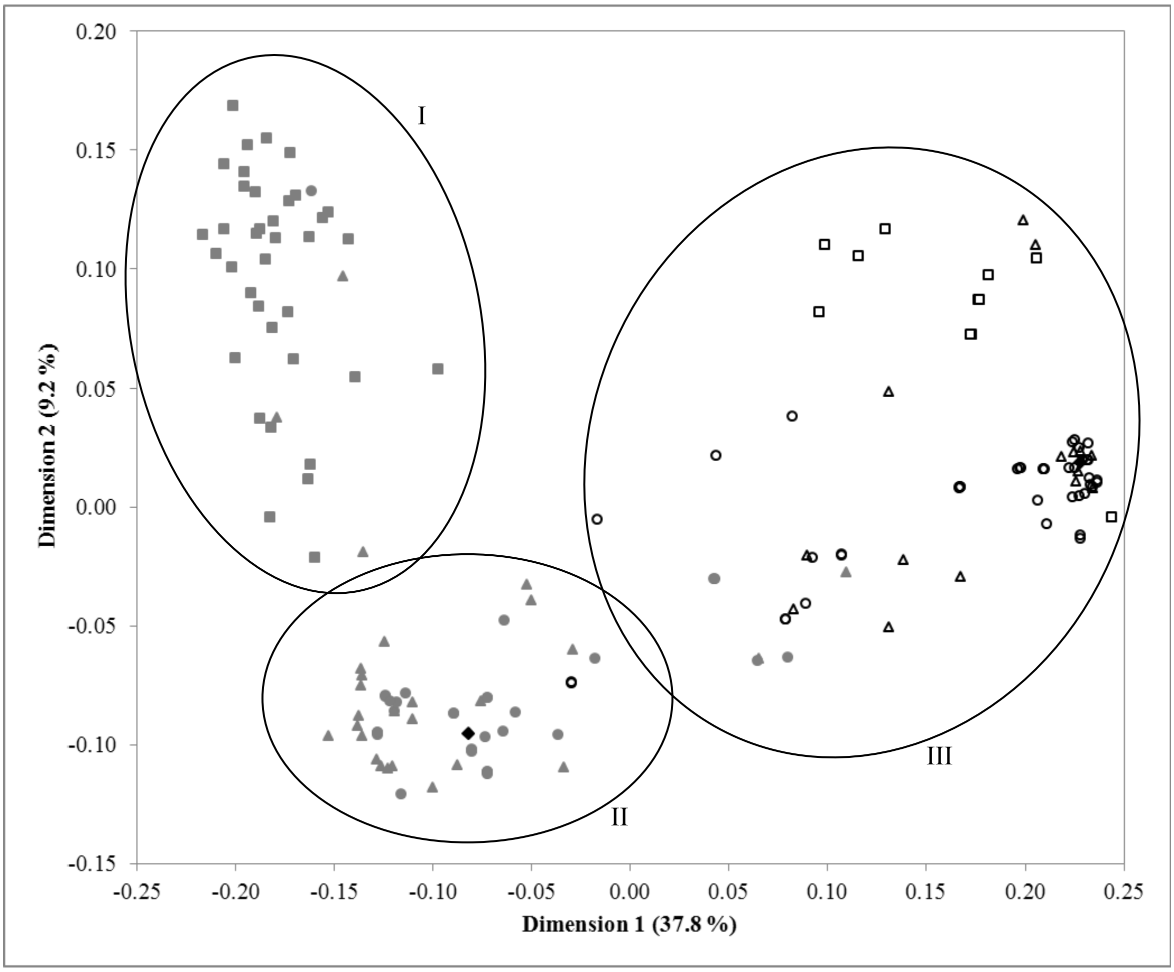


Fig 2 Principal coordinate analysis of 127 spring barley accessions based on all SNP markers. I, II and III indicates potential groups. Several landraces represented by multiple individuals. ○ 6 rowed landrace, △ 6 rowed cultivar from before 1975, □ 6 rowed cultivar from after 1975, ● 2 rowed landrace, ▲ 2 rowed cultivar from before 1975, ■ 2 rowed cultivar from after 1975 and ◆ 2 or 6 rowed segregating landrace.

Supplementary material

Manuscript 2

Online Resources 1

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Bor05135	Bor05135		BL	Finland	2		Boreal Plant Breeding
Bor09801	Bor09801		BL	Finland	6		Boreal Plant Breeding
NOS 15251-52	NOS 15251-52		BL	Denmark	2		Nordic Seed A/S Plant Breeding
NOS 16140-51	NOS 16140-51		BL	Denmark	2		Nordic Seed A/S Plant Breeding
NOS 17009-53	NOS 17009-53		BL	Denmark	2	expected 2014	Nordic Seed A/S Plant Breeding
Agneta	Agneta	NGB1508	CV	Sweden	6	1978	NordGen
Alabama	Alabama		CV	Germany	2	1999	BAR-OF project (ICROFS,Denmark)
Alf	Alf	NGB4707	CV	Denmark	2	1978	NordGen
Alliot	Alliot	NGB16757	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Amalika	Amalika		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Anakin	Anakin		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Anita	Anita	NGB15250	CV	Norway	6	1962	NordGen
Arla	Arla	NGB2681	CV	Sweden	2	1962	NordGen
Arve	Arve	NGB11311	CV	Norway	6	1990	NordGen
Åsa	Åsa	NGB4640	CV	Sweden	6	1949	NordGen
Birgitta	Birgitta	NGB1494	CV	Sweden	2	1963	NordGen
Birka	Birka	NGB4712	CV	Sweden	2	1981	NordGen
Brage	Brage		CV	Norway	6	2010	Graminor Plant Breeding
Brazil	Brazil		CV	France	2	2000	BAR-OF project (ICROFS,Denmark)
Brio	Brio	NGB9327	CV	Sweden	6	1924	NordGen
Calisi	Calisi		CV	Denmark	2	2013	Nordic Seed A/S Plant Breeding
Carlsberg	Carlsberg	NGB9442	CV	Denmark	2	1946	NordGen
Caruso	Caruso	NGB15059	CV	Denmark	2	1991	NordGen
Chevallier Tystofte	Chevallier Tystofte	NGB9443	CV	Denmark	2	1883	NordGen
Cicero	Cicero	NGB16756	CV	Denmark	2	1999	BAR-OF project (ICROFS,Denmark)
Columbus	Columbus		CV	Denmark	2	2009	Sejet Plant Breeding I/S
Culma	Culma		CV	Belgium	2	1997	BAR-OF project (ICROFS,Denmark)
Danpro	Danpro	NGB9659	CV	Denmark	2	1969	NordGen
Danuta	Danuta		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Denso Abed	Denso Abed	NGB8826	CV	Denmark	2	before 1965	NordGen
Drost Pajbjerg	Drost Pajbjerg	NGB6281	CV	Denmark	2	1951	NordGen
Edvin	Edvin		CV	Finland	6	2008	Boreal Plant Breeding

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Elmeri	Elmeri		CV	Finland	6	2009	Boreal Plant Breeding
Etu	Etu	NGB2679	CV	Finland	6	1970	NordGen
Eva	Eva	NGB1502	CV	Sweden	2	1973	NordGen
Evergreen	Evergreen		CV	Denmark	2	2010	Nordic Seed A/S Plant Breeding
Fabel Sejet	Fabel Sejet		CV	Denmark	2	2001	BAR-OF project (ICROFS,Denmark)
Fairytale	Fairytale		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Fero	Fero	NGB8231	CV	Denmark	2	1943	NordGen
Fløya	Fløya	NGB4664	CV	Norway	6	1939	NordGen
Fræg	Fræg	NGB8860	CV	Norway	6	1948	NordGen
Freja	Freja	NGB1485	CV	Sweden	2	1941	NordGen
Galant Carlberg	Galant Carlberg	NGB6308	CV	Denmark	2	1985	NordGen
Gaute	Gaute	NGB16732	CV	Norway	6	2000	Graminor Plant Breeding
Gunnar	Gunnar	NGB1515	CV	Sweden	2	1981	NordGen
Hafnia	Hafnia	NGB8878	CV	Denmark	2	1958	NordGen
Hannchen	Hannchen	NGB4617	CV	Sweden	2	1902	NordGen
Harbinger	Harbinger		CV	Finland	2	2009	Boreal Plant Breeding
Harriot	Harriot		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Helium	Helium		CV	Denmark	2	2001	Nordic Seed A/S Plant Breeding
Hydrogen	Hydrogen	NGB20346	CV	Denmark	2	2000	Nordic Seed A/S Plant Breeding
Ida	Ida	NGB2672	CV	Sweden	2	1979	NordGen
Iron	Iron		CV	Denmark	2	2007	Nordic Seed A/S Plant Breeding
Jacinta	Jacinta	NGB16665	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Jadar II	Jadar II	NGB457	CV	Norway	6	1947	NordGen
Jotun	Jotun	NGB4618	CV	Norway	6	1930	NordGen
Juli Abed	Juli Abed	NGB4585	CV	Denmark	6	1909	NordGen
Justus	Justus		CV	Finland	6	2013	Boreal Plant Breeding
Karin	Karin	NGB13021	CV	Sweden	6	1988	NordGen
Karri	Karri	NGB287	CV	Finland	2	1967	NordGen
Landora	Landora		CV	Germany	2	2000	BAR-OF project (ICROFS,Denmark)
Laurikka	Laurikka		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Lavrans	Lavrans	NGB16727	CV	Norway	6	1999	Graminor Plant Breeding
Linus	Linus	NGB13482	CV	Sweden	2	1997	NordGen
Lysimax	Lysimax	NGB15055	CV	Denmark	2	1994	NordGen
Mari	Mari	NGB1491	CV	Sweden	2	1960	NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Møyjar	Møyjar	NGB2106	CV	Norway	2	1969	NordGen
Nord	Nord	NGB9942	CV	Finland	6	1988	NordGen
Odin	Odin	NGB16755	CV	Denmark	2	1981	NordGen
Orthega	Orthega		CV	Germany	2	1997	BAR-OF project (ICROFS,Denmark)
Otira	Otira	NGB16752	CV	Denmark	2	1997	BAR-OF project (ICROFS,Denmark)
Paavo	Paavo	NGB13661	CV	Finland	6	1959	NordGen
Pallas	Pallas		CV	Sweden	2	1970	BAR-OF project (ICROFS,Denmark)
Piikkiönohra	Piikkiönohra	NGB8234	CV	Finland	2	1922	NordGen
Pinocchio	Pinocchio		CV	Denmark	2	2011	Sejet Plant Breeding I/S
Pirkka	Pirkka	NGB292	CV	Finland	6	1952	NordGen
Prestige	Prestige	NGB16750	CV	France	2	2000	The Danish AgriFish Agency, Tystofte
Prominant	Prominant	NGB15066	CV	Denmark	2	1999	NordGen
Punto	Punto	NGB23207	CV	Denmark	2	1995	BAR-OF project (ICROFS,Denmark)
Rex Abed	Rex Abed	NGB9465	CV	Denmark	2	1913	NordGen
Sebastian	Sebastian		CV	Denmark	2	2002	NordGen
Severi	Severi		CV	Finland	6	2013	Boreal Plant Breeding
Simba	Simba		CV	Denmark	2	2002	NordGen
Stange	Stange	NGB2109	CV	Norway	2	1978	NordGen
Tammi	Tammi	NGB6925	CV	Finland	6	1937	NordGen
Tore	Tore	NGB6605	CV	Norway	6	1986	NordGen
Trekker	Trekker		CV	Finland	2	2013	Boreal Plant Breeding
Visir	Visir	NGB1496	CV	Sweden	2	1971	NordGen
Zita	Zita	NGB7236	CV	Denmark	2	1974	NordGen
Anita Högsby-korn	Anita Högsby-korn	NGB263	L	Sweden	2		NordGen
Bjørne	Bjørne	NGB9326	L		6		NordGen
Bryssel	Bryssel KVL 28	NGB8222	L	Belgium	6		NordGen
Cluj	Cluj KVL 100	NGB9355	L	Romania	6		NordGen
Dønnes	Dønnes	NGB9448	L	Norway	6		NordGen
Grenoble	Grenoble I KVL 131	NGB9378	L	France	6		NordGen
Griechische	Griechische KVL 56	NGB9333	L	Greece	6		NordGen
Hannuksela	Hannuksela	NGB325	L	Finland	6		NordGen
Junkkari	Junkkari	NGB307	L	Finland	6		NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Kilpau	Kilpau ME0201	NGB1159	L	Finland	6	19810819	NordGen
Königsberg	Königsberg KVL 18	NGB9310	L		6		NordGen
Langaks	Langaks	NGB6300	L	Faroe Islands	6	196108--	NordGen
Laukko	Laukko	NGB16881	L	Finland	2		NordGen
Ljubljana KVL 15	Ljubljana KVL 15	NGB9308	L	Croatia	6		NordGen
Ljubljana KVL 395	Ljubljana KVL 395	NGB6941	L	Croatia	2		NordGen
Luusua	Luusua EH0401	NGB792	L	Finland	6	19790907	NordGen
Lynderupgaard	Lynderupgaard	NGB9529	L	Denmark	6	195205--	NordGen
Magdeburg	Magdeburg KVL 358	NGB9485	L	Germany	2		NordGen
Metz	Metz KVL 124	NGB9373	L	France	6		NordGen
Montpellier	Montpellier KVL 209	NGB9410	L	France	2		NordGen
Nordslesvigsk Kæmpe	Nordslesvigsk Kæmpe	NGB9339	L	Denmark	6	1890	NordGen
Nue Grosse	Nue Grosse	NGB9436	L	Denmark	2		NordGen
Oppdal	Oppdal	NGB13670	L	Norway	6		NordGen
Osiris	Osiris J-1277	NGB9639	L		6		NordGen
Oslo	Oslo KVL 25	NGB9315	L	Norway	6		NordGen
Pavia	Pavia KVL 386	NGB9501	L	Italy	2		NordGen
Probstei	Probstei/Tabor KVL 362	NGB9487	L	Czech Republic	2		NordGen
Rauto	Rauto	NGB265	L	Finland	2;6		NordGen
Rehakka	Rehakka-65	NGB314	L	Finland	2		NordGen
Sarkalahti	Sarkalahti ME0103	NGB27	L	Finland	6	19800810	NordGen
Solenbyg	Solenbyg	NGB13402	L	Norway	6		NordGen
Sort Glatstakket	Sort Glatstakket	NGB9345	L		6		NordGen
Stjernebyg fra Færøerne	Stjernebyg fra Færøerne	NGB4701	L	Faroe Islands	6	196908--	NordGen
Szeged	Szeged KVL 347	NGB9478	L	Hungary	2		NordGen
Tartu	Tartu KVL 349	NGB9479	L	Estonia	2		NordGen
Vilm	Vilm KVL 248	NGB9435	L	Germany	2		NordGen
Vilmorin	Vilmorin KVL 126	NGB9375	L		6		NordGen
Ylenjoki	Ylenjoki AP0301	NGB4413	L	Finland	2;6	19830825	NordGen

Accession name	Location / Breeder institute	Culton pedigree
Bor05135	Boreal Plant Breeding	
Bor09801	Boreal Plant Breeding	
NOS 15251-52	Nordic Seed A/S Plant Breeding	
NOS 16140-51	Nordic Seed A/S Plant Breeding	
NOS 17009-53	Nordic Seed A/S Plant Breeding	Iron x Keops
Agneta	Sveriges Utsadesforening Svalöf	(Åsa x Frisia) ^2 x (Edda II ^4 x Monte Cristo)
Alabama	KWS SAAT AG	(Ml-i x 2.51784) x Krona
Alf	Nordic Seed A/S Plant Breeding	Mutant in Bomi
Alliot	Nordic Seed A/S Plant Breeding	Waggon*Imidis
Amalika	Nordic Seed A/S Plant Breeding	NOS 1184-07 x Quench
Anakin	Sejet Plant Breeding I/S	Tumbler x Respons
Anita	Norwegian Agrcultural University	HOL-44 x Arve; / (Asplund x Ds295) x Varde
Arla	Weibullsholm Plant Breeding Institute	(Maja x ((Hanna x Svanhals) x Opal)) x Tammi
Arve	Graminor Plant Breeding	(Otra x Vigdis) x Agneta
Åsa	Sveriges Utsadesforening Svalöf	Dore x Vega
Birgitta	Sveriges Utsadesforening Svalöf	(Opal x Vega) x Maja
Birka	Weibullsholm Plant Breeding Institute	W 82-68 x W 17-68
Brage	Graminor Plant Breeding	Lavrans/NK91650
Brazil	KWS SAAT AG	Trebon x Cooper
Brio	Sveriges Utsadesforening Svalöf	Selection from 6-row barley from Skåne
Calisi	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Carlsberg	Carlsberg	Prentice x Maja
Caruso	Carlsberg	(Rupal X Terrax) X Grit
Chevallier Tystofte		
Cicero	Sejet Plant Breeding I/S	Chalice x SJ 933275
Columbus	Sejet Plant Breeding I/S	Isabella x Publican
Culma	Limagrain	590907 x Hart
Danpro	Carlsberg	Proctor x Dana
Danuta	NordSaat	90014DH x (Salome x Maresi)
Denso Abed	The Abed Foundation	Rigel
Drost Pajbjerg	The Pajbjerg Foundation	Maja x Kenia
Edvin	Boreal Plant Breeding	Verner/Hja 85194
Elmeri	Boreal Plant Breeding	Thule/Verner

Accession name	Location / Breeder institute	Culton pedigree
Etu	Agricultural Research Centre	Bonus M x Varde
Eva	Sveriges Utsadesforening Svalöf	Birgitta x Mari
Evergreen	Nordic Seed A/S Plant Breeding	Br6920b115*Quench
Fabel Sejet	Sejet Plant Breeding I/S	Newgrange x SJ 2256
Fairytale	Sejet Plant Breeding I/S	Colston x (Recept x Power)
Fero	Øtofte	Selection from Kenia
Fløya	(SFL)Norwegian Government Res.Station Holt	Selection from Ørnesbygg
Fræg	(SFL)Norwegian Government Res.Station Holt	Asplund x Maskin
Freja	Sveriges Utsadesforening Svalöf	Seger x Opal
Galant Carlberg	Carlsberg	Mutation in Triumph
Gaute	Graminor Plant Breeding	SvN82114/Vo13647-77
Gunnar	Svalöf AB	Kristina x ((Mari 6 x 57/510-44) x Å 61718)
Hafnia		Freja x Lenta
Hannchen	Sveriges Utsadesforening Svalöf	Line selection from Hanna
Harbinger	Boreal Plant Breeding	
Harriot	Nordsaat	(GS 1635 x Nordus) x Annabell
Helium	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Hydrogen	Nordic Seed A/S Plant Breeding	(Alis x Digger) x Derkado
Ida	Weibullsholm Plant Breeding Institute	Tellus x Arla M1
Iron	Nordic Seed A/S Plant Breeding	Marnie x LP 813.6.98
Jacinta	Nordic Seed A/S Plant Breeding	(Alexis x Meltan) x Canut
Jadar II	(SFL)Norwegian Government Res.Station Forus	Jadar x Asplund
Jotun	(SLF)Norwegian Government Res.Station Løken	Selection from the landrace Oppdalbygg
Juli Abed	The Abed Foundation	Collected material
Justus	Boreal Plant Breeding	
Karin	Svalöf AB	(Åsa x Edda II) x Varde
Karri	Hankkija Plant Breeding Station	Carlsberg x Rigel
Landora	Saatzucht Hadmersleben GmbH	Cross breeding (Hadm86508-91 x Hadm46544-88)
Laurikka	Nordic Seed A/S Plant Breeding	(Ceb 7931 x Pompadour) x (577223 x Golf)
Lavrans	Graminor Plant Breeding	Vera//Arve/H82009-1-3
Linus	Svalöf Weibull AB	WW 7749 x Ariel
Lysimax	Sejet Plant Breeding I/S	Ca 040223 x Carula
Mari	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Møyjar	(SFL)Norwegian Government Res.Station Møystad	Domen x Herta

Accession name	Location / Breeder institute	Culton pedigree
Nord	Lantbrukets Forskningscentrals Växtförädlingsanstalt	Otra x Etu
Odin	Carlsberg	Sv 66433 x ALL.297
Orthega	KWS SAAT AG	Tucson x Quench
Otira	Sejet Plant Breeding I/S	Bartok x Sj 930331
Paavo		(Tammi x Gull) x O.A.C. 21
Pallas	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Piikkiönohra		Line from landrace from Piikkiö, Southwest Finlar
Pinocchio	Sejet Plant Breeding I/S	Quench x SJ Afrodite
Pirkka	Hankkija Plant Breeding Station	TA 04369 x TA 05864
Prestige	RAGT	AC 00/767/5 x Anakin
Prominant	Sejet Plant Breeding I/S	Caminant x Vintage
Punto	Sejet Plant Breeding I/S	Lamba x Meltan
Rex Abed	The Abed Foundation	Selection from old Danish 2-row
Sebastian	Sejet Plant Breeding I/S	Lux x Viskosa
Severi	Boreal Plant Breeding	
Simba	Sejet Plant Breeding I/S	Otira x Prolog
Stange	Norwegian Government Res.Station Møystad	Mari x Ingrid
Tammi	Hankkija Plant Breeding Station	Asplund x Olli
Tore	Norwegian Agrcultural University /(Graminor)	Lise x Clermont
Trekker	Boreal Plant Breeding	
Visir	Sveriges Utsadesforening Svalöf	Pallas x Long Glumes
Zita	The Pajbjerg Foundation	PF 203/7748 x Vada
Anita Högsby-korn		
Bjørne		
Bryssel KVL 28		
Cluj KVL 100		
Dønnes		
Grenoble I KVL 131		
Griechische KVL 56		
Hannuksela		
Junkkari		
Kilpau ME0201	Kilpau, Oulainen	
Königsberg KVL 18		

Accession name	Location / Breeder institute	Culton pedigree
Langaks	Faroe Islands	
Laukko		
Ljubljana KVL 15		
Ljubljana KVL 395		
Luusua EH0401	Luusua, Kemijärvi	
Lynderupgaard	Lyderupgaard	
Magdeburg KVL 358		
Metz KVL 124		
Montpellier KVL 209		
Nordslesvigsk Kæmpe		
Nue Grosse		
Oppdal		
Osiris J-1277		
Oslo KVL 25		
Pavia KVL 386		
Probstei/Tabor KVL 362		
Rauto	(SFL)Norwegian Government Res.Station Løken	
Rehakka-65		
Sarkalahti ME0103	Sarkalahti, Luumäki	
Solenbyg		
Sort Glatstakket		
Stjernebyg fra Færøerne	Faroe Islands	
Szeged KVL 347		
Tartu KVL 349		
Vilm KVL 248		
Vilmorin KVL 126		
Ylenjoki AP0301	Jussila, Ylenjoki, Valkeakoski	

Online Resources 2

	Q-value				
Acccession name	Group 1	Group 2	Group 3	Predominant group	Group
Culma	0.908	0.091	0.000	0.908	1
Alabama	0.651	0.349	0.000	0.651	1
Cicero	0.935	0.065	0.000	0.935	1
Fabel Sejet	0.864	0.135	0.000	0.864	1
Otira	0.887	0.081	0.032	0.887	1
Punto	0.563	0.436	0.000	0.563	1
Sebastian	1.000	0.000	0.000	1.000	1
Odin	0.783	0.149	0.068	0.783	1
Galant Carlberg	0.668	0.332	0.000	0.668	1
Prominant	0.844	0.156	0.000	0.844	1
Evergreen	1.000	0.000	0.000	1.000	1
Iron	0.979	0.001	0.020	0.979	1
Helium	0.892	0.108	0.000	0.892	1
Hydrogen	0.649	0.351	0.001	0.649	1
Jacinta	0.861	0.139	0.000	0.861	1
Alliot	0.866	0.134	0.000	0.866	1
Amalika	0.932	0.067	0.001	0.932	1
Laurikka	0.999	0.000	0.000	0.999	1
NOS 15251-52	0.959	0.041	0.000	0.959	1
NOS 16140-51	1.000	0.000	0.000	1.000	1
NOS 17009-53	0.930	0.069	0.001	0.930	1
Calisi	1.000	0.000	0.000	1.000	1
Anakin	0.919	0.080	0.000	0.919	1
Columbus	1.000	0.000	0.000	1.000	1
Simba	0.997	0.002	0.000	0.997	1
Fairytale	0.999	0.001	0.000	0.999	1
Pinocchio	1.000	0.000	0.000	1.000	1
Harbinger	0.913	0.086	0.001	0.913	1
Bor05135	0.922	0.078	0.000	0.922	1
Trekker	0.996	0.003	0.001	0.996	1
Brazil	0.961	0.039	0.000	0.961	1
Prestige	0.936	0.064	0.000	0.936	1
Danuta	0.679	0.321	0.000	0.679	1
Harriot	0.998	0.001	0.000	0.998	1
Landora	0.747	0.253	0.000	0.747	1
Orthega	0.561	0.320	0.119	0.561	1
Linus	0.547	0.450	0.003	0.547	1
Pavia KVL 386	0.986	0.003	0.010	0.986	1
Chevallier Tystofte	0.000	0.892	0.108	0.892	2
Rex Abed	0.001	0.920	0.078	0.920	2
Fero	0.001	0.984	0.015	0.984	2
Carlsberg	0.003	0.997	0.000	0.997	2
Drost Pajbjerg	0.017	0.983	0.000	0.983	2
Hafnia	0.078	0.921	0.001	0.921	2
Danpro	0.001	0.996	0.003	0.996	2
Zita	0.334	0.665	0.001	0.665	2
Alf	0.047	0.953	0.000	0.953	2
Caruso	0.476	0.524	0.000	0.524	2
Lysimax	0.362	0.637	0.000	0.637	2
Denso Abed	0.002	0.998	0.000	0.998	2
Piikkiönohra	0.001	0.511	0.488	0.511	2

	Q-value				
Acccession name	**Group 1**	**Group 2**	**Group 3**	**Predominant group**	**Group**
Karri	0.001	0.999	0.000	0.999	2
Møyjar	0.065	0.933	0.001	0.933	2
Stange	0.010	0.989	0.001	0.989	2
Hannchen	0.002	0.971	0.027	0.971	2
Freja	0.001	0.999	0.000	0.999	2
Mari	0.000	1.000	0.000	1.000	2
Birgitta	0.006	0.816	0.179	0.816	2
Visir	0.000	1.000	0.000	1.000	2
Eva	0.001	0.797	0.203	0.797	2
Ida	0.114	0.716	0.170	0.716	2
Gunnar	0.001	0.915	0.084	0.915	2
Birka	0.109	0.869	0.023	0.869	2
Pallas	0.000	1.000	0.000	1.000	2
Königsberg KVL 18	0.081	0.658	0.261	0.658	2
Sort Glatstakket	0.081	0.658	0.261	0.658	2
Ljubljana KVL 395	0.247	0.576	0.177	0.576	2
Probstei/Tabor KVL 362	0.000	0.999	0.000	0.999	2
Probstei/Tabor KVL 362	0.000	0.999	0.001	0.999	2
Probstei/Tabor KVL 362	0.000	0.999	0.001	0.999	2
Probstei/Tabor KVL 362	0.000	0.999	0.001	0.999	2
Tartu KVL 349	0.001	0.929	0.070	0.929	2
Tartu KVL 349	0.001	0.930	0.070	0.930	2
Laukko	0.087	0.774	0.139	0.774	2
Laukko	0.091	0.655	0.254	0.655	2
Rauto	0.001	0.959	0.040	0.959	2
Rehakka-65	0.001	0.511	0.488	0.511	2
Grenoble I KVL 131	0.141	0.568	0.291	0.568	2
Montpellier KVL 209	0.070	0.498	0.431	0.498	2
Montpellier KVL 209	0.070	0.498	0.432	0.498	2
Magdeburg KVL 358	0.001	0.999	0.000	0.999	2
Magdeburg KVL 358	0.001	0.999	0.000	0.999	2
Magdeburg KVL 358	0.001	0.998	0.001	0.998	2
Magdeburg KVL 358	0.001	0.999	0.000	0.999	2
Magdeburg KVL 358	0.001	0.999	0.000	0.999	2
Magdeburg KVL 358	0.001	0.999	0.000	0.999	2
Vilm KVL 248	0.121	0.767	0.112	0.767	2
Vilm KVL 248	0.123	0.766	0.111	0.766	2
Szeged KVL 347	0.001	0.930	0.069	0.930	2
Szeged KVL 347	0.001	0.929	0.070	0.929	2
Pavia KVL 386	0.002	0.998	0.000	0.998	2
Pavia KVL 386	0.001	0.998	0.000	0.998	2
Pavia KVL 386	0.002	0.998	0.000	0.998	2
Pavia KVL 386	0.000	0.999	0.000	0.999	2
Pavia KVL 386	0.001	0.999	0.000	0.999	2
Anita Högsby-korn	0.001	0.939	0.060	0.939	2
Anita Högsby-korn	0.001	0.939	0.060	0.939	2
Anita Högsby-korn	0.001	0.893	0.106	0.893	2
Anita Högsby-korn	0.001	0.940	0.060	0.940	2
Anita Högsby-korn	0.001	0.844	0.155	0.844	2
Juli Abed	0.000	0.117	0.882	0.882	3
Tammi	0.000	0.000	1.000	1.000	3
Pirkka	0.001	0.012	0.987	0.987	3

	Q-value				
Acccession name	**Group 1**	**Group 2**	**Group 3**	**Predominant group**	**Group**
Paavo	0.001	0.295	0.704	0.704	3
Etu	0.001	0.468	0.531	0.531	3
Nord	0.000	0.190	0.810	0.810	3
Elmeri	0.332	0.089	0.580	0.580	3
Edvin	0.293	0.093	0.615	0.615	3
Justus	0.268	0.188	0.544	0.544	3
Severi	0.333	0.003	0.664	0.664	3
Jotun	0.000	0.000	1.000	1.000	3
Fløya	0.000	0.000	1.000	1.000	3
Jadar II	0.010	0.003	0.987	0.987	3
Fræg	0.000	0.000	1.000	1.000	3
Anita	0.000	0.233	0.766	0.766	3
Tore	0.158	0.223	0.620	0.620	3
Arve	0.114	0.000	0.886	0.886	3
Lavrans	0.182	0.000	0.818	0.818	3
Gaute	0.179	0.002	0.819	0.819	3
Brage	0.183	0.018	0.799	0.799	3
Brio	0.001	0.001	0.998	0.998	3
Åsa	0.000	0.000	1.000	1.000	3
Arla	0.001	0.409	0.590	0.590	3
Agneta	0.136	0.000	0.863	0.863	3
Karin	0.158	0.000	0.841	0.841	3
Bor09801	0.026	0.179	0.795	0.795	3
Bjørne	0.000	0.001	0.998	0.998	3
Königsberg KVL 18	0.005	0.238	0.757	0.757	3
Königsberg KVL 18	0.004	0.239	0.757	0.757	3
Osiris J-1277	0.221	0.276	0.503	0.503	3
Vilmorin KVL 126	0.000	0.000	1.000	1.000	3
Vilmorin KVL 126	0.000	0.000	1.000	1.000	3
Bryssel KVL 28	0.000	0.000	1.000	1.000	3
Bryssel KVL 28	0.000	0.000	1.000	1.000	3
Ljubljana KVL 15	0.000	0.000	0.999	0.999	3
Ljubljana KVL 15	0.000	0.000	0.999	0.999	3
Ljubljana KVL 15	0.000	0.000	0.999	0.999	3
Lynderupgaard	0.000	0.000	0.999	0.999	3
Lynderupgaard	0.001	0.032	0.967	0.967	3
Nordslesvigsk Kæmpe	0.000	0.024	0.975	0.975	3
Nordslesvigsk Kæmpe	0.000	0.024	0.976	0.976	3
Nue Grosse	0.240	0.356	0.405	0.405	3
Langaks	0.053	0.084	0.864	0.864	3
Langaks	0.053	0.084	0.863	0.863	3
Stjernebyg fra Færøerne	0.052	0.083	0.864	0.864	3
Luusua EH0401	0.000	0.000	1.000	1.000	3
Luusua EH0401	0.000	0.000	1.000	1.000	3
Sarkalahti ME0103	0.006	0.001	0.992	0.992	3
Sarkalahti ME0103	0.000	0.000	1.000	1.000	3
Kilpau ME0201	0.000	0.000	1.000	1.000	3
Kilpau ME0201	0.000	0.000	1.000	1.000	3
Ylenjoki AP0301	0.000	0.000	0.999	0.999	3
Hannuksela	0.000	0.000	1.000	1.000	3
Junkkari	0.000	0.000	1.000	1.000	3
Junkkari	0.000	0.000	1.000	1.000	3

	Q-value				
Acccession name	**Group 1**	**Group 2**	**Group 3**	**Predominant group**	**Group**
Rehakka-65	0.001	0.481	0.519	0.519	3
Grenoble I KVL 131	0.000	0.000	1.000	1.000	3
Metz KVL 124	0.054	0.408	0.538	0.538	3
Griechische KVL 56	0.059	0.378	0.564	0.564	3
Griechische KVL 56	0.059	0.377	0.564	0.564	3
Dønnes	0.037	0.444	0.519	0.519	3
Oppdal	0.001	0.001	0.999	0.999	3
Oslo KVL 25	0.004	0.239	0.757	0.757	3
Oslo KVL 25	0.003	0.239	0.757	0.757	3
Solenbyg	0.000	0.000	1.000	1.000	3
Cluj KVL 100	0.001	0.495	0.504	0.504	3
Cluj KVL 100	0.001	0.495	0.504	0.504	3

Online Resources 3

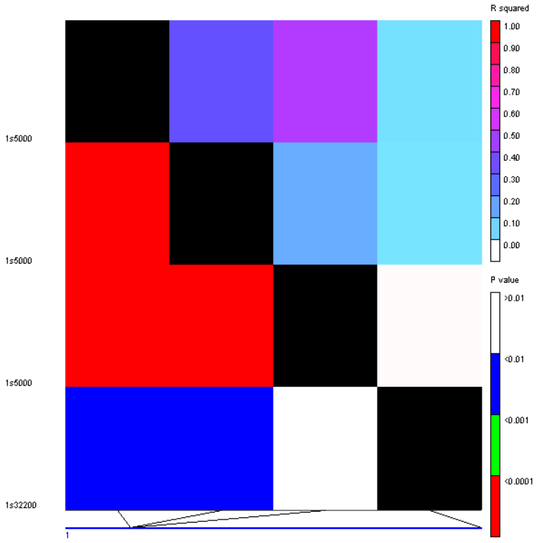
Linkage group	9k iSELECT Marker Nam	Chrom	Pos (cM)	log_{10}(P) MwP	Eff.	SD.eff	Treatment	Phenotype
1	i_11_10419	1H	5.0	3.348	0.425	1.474	amb	ET
1	i_11_10419	1H	5.0	3.423	0.456	1.411	amb	EG
1	i_SCRI_RS_232577	1H	5.0	4.322	2.687	8.400	W^2	EG
1	i_SCRI_RS_60293	1H	5.0	3.166	-0.633	1.363	amb	EG
2	i_12_31276	1H	32.2	3.121	-1.053	2.936	S^2	GY
3	i_SCRI_RS_143250	2H	23.0	3.867	0.438	1.197	tmp	GY
3	i_11_21015	2H	23.2	3.119	-0.432	1.209	tmp	GY
3	i_11_21015	2H	23.2	3.156	-0.599	1.609	tmp	ΔGY
3	i_SCRI_RS_120529	2H	23.2	2.975	-0.423	1.208	tmp	GY
3	i_SCRI_RS_120529	2H	23.2	3.043	-0.605	1.608	tmp	ΔGY
3	i_SCRI_RS_12516	2H	23.8	3.442	0.434	1.199	tmp	GY
4	i_12_30657	2H	39.7	3.180	-0.647	1.688	tmp	BM
4	i_12_30657	2H	39.7	3.972	-0.804	1.907	tmp	ΔBM
5	i_11_20667	2H	66.3	3.195	-0.862	1.420	amb	EG
5	i_SCRI_RS_6727	2H	66.9	3.236	-1.173	1.910	CO_2	EG
5	i_SCRI_RS_6727	2H	66.9	3.825	-1.328	2.172	CO_2	ET
5	i_SCRI_RS_6727	2H	66.9	5.578	-1.332	2.864	tmp	ET
5	i_SCRI_RS_73	2H	67.9	3.095	1.059	1.804	O_3	EG
5	i_SCRI_RS_15537	2H	106.9	3.442	0.994	1.790	O_3	EG
5	i_11_11236	2H	107.0	3.242	-0.783	1.780	O_3	EG
6	i_11_10092	2H	113.5	3.096	0.686	1.611	tmp	ΔGY
6	i_SCRI_RS_129178	2H	114.1	3.265	-0.706	1.817	×2	ΔET
7	i_SCRI_RS_167825	3H	100.3	3.612	0.626	1.387	O_3	GY
8	i_SCRI_RS_144313	3H	135.5	3.260	0.120	0.280	tmp	ΔHI
8	i_SCRI_RS_144313	3H	135.5	3.390	0.762	1.673	tmp	ΔGY
8	i_SCRI_RS_144313	3H	135.5	4.345	23.213	40.098	tmp	ΔGN
9	i_12_30992	4H	43.3	3.319	0.668	1.360	amb	BM
9	i_12_30992	4H	43.3	3.964	-0.611	1.402	O_3	ΔBM
9	i_12_10371	4H	43.5	3.518	-20.228	33.809	amb	GN
9	i_11_20180	4H	43.8	3.518	20.228	33.809	amb	GN
10	i_12_30564	4H	49.7	3.867	-0.846	2.037	CO_2	ΔGY
10	i_12_30564	4H	49.7	4.874	-12.941	34.971	CO_2	ΔGN

Linkage group	9k iSELECT Marker Nam	Chrom	Pos (cM)	log_{10}(P) MwP	Eff.	SD.eff	Treatment	Phenotype
10	i_11_11405	4H	49.9	3.867	-0.846	2.037	CO_2	ΔGY
10	i_11_11405	4H	49.9	4.874	-12.941	34.971	CO_2	ΔGN
10	i_SCRI_RS_222133	4H	49.9	3.867	0.846	2.037	CO_2	ΔGY
10	i_SCRI_RS_222133	4H	49.9	4.874	12.941	34.971	CO_2	ΔGN
10	i_11_20178	4H	83.6	4.326	-0.534	1.566	O_3	ΔGY
11	i_SCRI_RS_192689	4H	104.0	3.182	-0.030	0.081	W^2	HI
12	i_11_20553	5H	0.1	4.010	-1.088	1.819	O_3	EG
13	i_11_11048	5H	23.6	3.199	-0.347	1.339	O_3	ΔBM
14	i_SCRI_RS_144841	5H	50.0	4.054	0.088	0.177	×2	HI
15	i_SCRI_RS_162696	5H	113.9	3.442	-0.092	0.226	tmp	HI
16	i_SCRI_RS_166857	5H	128.1	3.109	0.072	0.222	tmp	HI
17	i_11_10536	5H	144.5	2.976	-3.898	8.678	W^2	EG
18	i_11_20996	6H	88.6	3.597	-3.010	6.834	W^2	BM
19	i_SCRI_RS_8034	6H	100.4	3.941	0.053	0.091	W^2	HI
19	i_11_20036	6H	100.8	3.272	-0.746	2.074	tmp	EG
20	i_SCRI_RS_93773	7H	0.3	3.409	10.653	30.216	O3	GN
21	i_SCRI_RS_213333	7H	21.4	2.966	-11.972	29.538	×2	ΔGN
22	i_12_30880	7H	54.4	4.319	1.305	2.189	CO_2	GY
22	i_12_30880	7H	54.4	5.037	1.317	2.195	CO_2	ΔGY
22	i_12_30880	7H	54.4	5.274	19.371	37.005	CO_2	ΔGN
22	i_SCRI_RS_230478	7H	54.8	3.502	-1.963	5.213	W^2	GY
22	i_SCRI_RS_229041	7H	55.0	3.739	15.339	35.964	CO2	ΔGN
22	i_SCRI_RS_229041	7H	55.0	3.756	1.101	2.129	CO2	GY
23	i_SCRI_RS_204256	7H	91.2	3.702	0.968	1.769	tmp	BM
24	i_SCRI_RS_107367	7H	108.1	3.547	-0.065	0.168	O_3	HI
25	i_SCRI_RS_1383	7H	120.0	3.042	-0.012	0.043	S^2	HI
25	i_SCRI_RS_1383	7H	120.0	3.248	0.061	0.222	tmp	HI
25	i_SCRI_RS_140746	7H	120.4	3.665	-0.870	2.910	S^2	GY

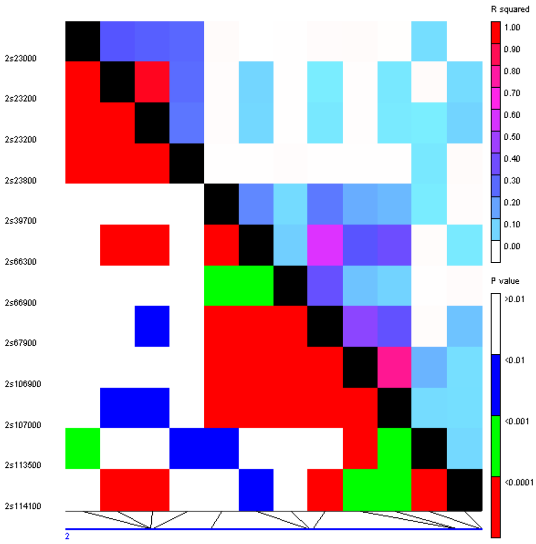
Online Resources 4

LD analysis of associated markers on each chromosome.

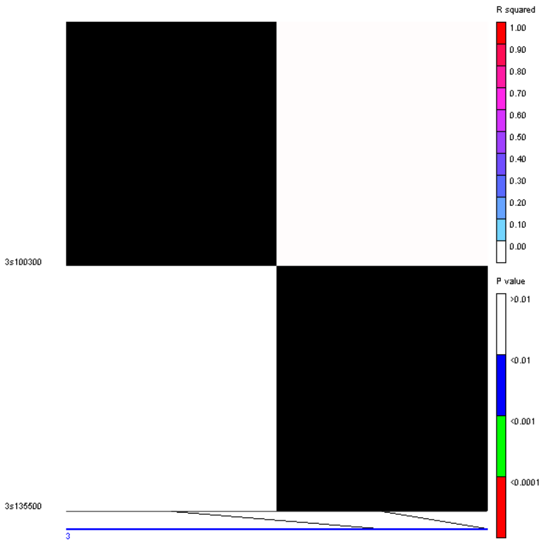
9k_iSELECT_Marker_Name	Chrom.	Pos.
i_11_10419	1H	5.0
i_SCRI_RS_232577	1H	5.0
i_SCRI_RS_60293	1H	5.0
i_12_31276	1H	32.2



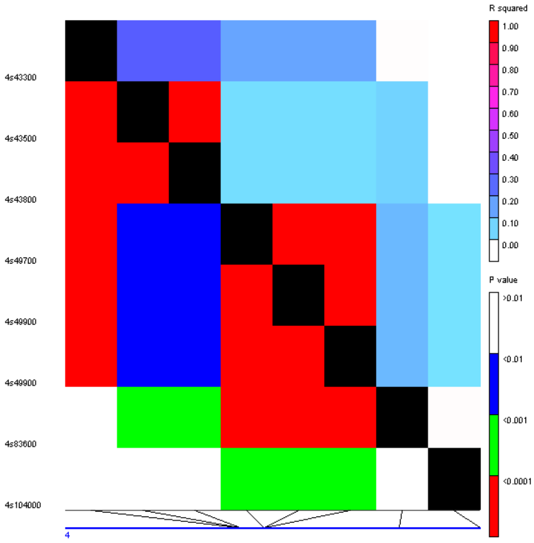
9k_iSELECT_Marker_Name	Chrom.	Pos.
i_SCRI_RS_143250	2H	23.0
i_11_21015	2H	23.2
i_SCRI_RS_120529	2H	23.2
i_SCRI_RS_12516	2H	23.8
i_12_30657	2H	39.7
i_11_20667	2H	66.3
i_SCRI_RS_6727	2H	66.9
i_SCRI_RS_73	2H	67.9
i_SCRI_RS_15537	2H	106.9
i_11_11236	2H	107.0
i_11_10092	2H	113.5
i_SCRI_RS_129178	2H	114.1



9k_iSELECT_Marker_Name	Chrom.	Pos.
i_SCRI_RS_167825	3H	100.3
i_SCRI_RS_144313	3H	135.5



9k_iSELECT_Marker_Name	Chrom.	Pos.
i_12_30992	4H	43.3
i_12_10371	4H	43.5
i_11_20180	4H	43.8
i_12_30564	4H	49.7
i_11_11405	4H	49.9
i_SCRI_RS_222133	4H	49.9
i_11_20178	4H	83.6
i_SCRI_RS_192689	4H	104.0



9k_iSELECT_Marker_Name	Chrom.	Pos.
i_11_20553	5H	0.1
i_11_11048	5H	23.6
i_SCRI_RS_144841	5H	50.0
i_SCRI_RS_162696	5H	113.9
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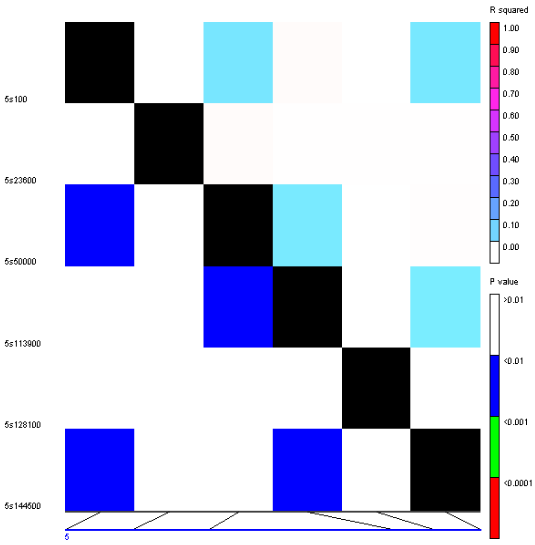

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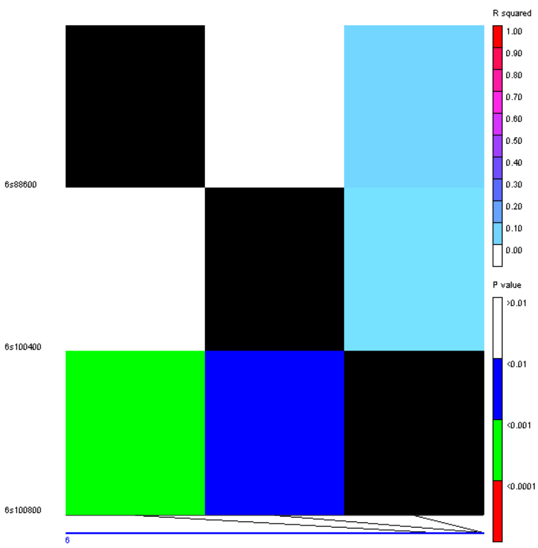

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i_SCRI_RS_204256	7H	91.2
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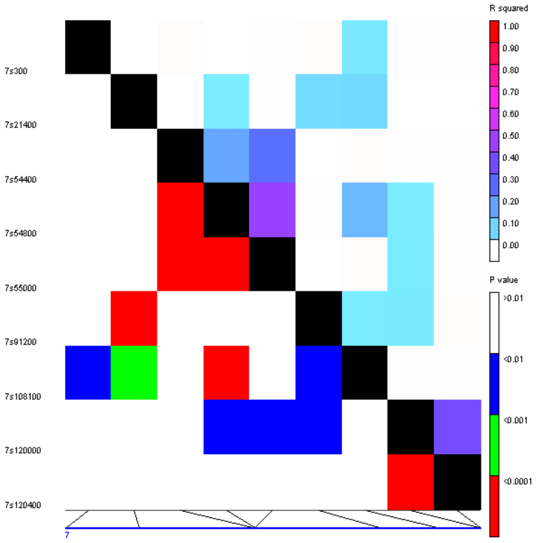

Manuscript 3

Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 barley (*Hordeum vulgare* L.) accessions

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KEYWORDS: *Hordeum vulgare, climate change, grain protein harvested (GPH), near-infrared spectroscopy, tropospheric ozone, spring barley*

ABSTRACT: Climate change is predicted to decrease future grain yields and influence grain protein concentration (GPC). In the present study a diverse set of 108 spring barley accessions were cultivated under predicted future levels of temperature, [CO_2] and [O_3] as single-factors and temperature and [CO_2] in combination (IPCC SRES scenario A1FI). We found, that the response in GPC from the single-factor treatments - 29 % increased under elevated temperature and 5 % decreased under elevated [CO_2] - could not be used to predict the 8 % increase in GPC in the combined treatment. Ozone as single factor increased grain protein with 6 %. In a future scenario with lowered grain yield, harvesting as much protein as possible seems desirable. Grain protein harvested (GPH) only increased under elevated [CO_2] and was lowered 23 % in the future climate scenario of elevated temperature and [CO_2]. Vast variation in the response of the 108 accessions was identified. This variation should be further exploited to increase the grain protein harvested under future climate change conditions.

INTRODUCTION

Climate change, with increased atmospheric concentration of the greenhouse gasses carbon dioxide ([CO_2]) and ozone ([O_3]) together with rising temperature, is likely to decrease plant production in the future and influence grain protein and quality[1–5]. According to the latest projections by IPCC (Intergovernmental Panel of Climate Change) climatic conditions point to the worst-case scenario (RCP8.5) unless actions are taken in the near future[6]. In the worst-case scenario temperature is predicted to rise 5 °C and [CO_2] to reach 1000 ppm compared to the 400 ppm of today. The increase of [O_3] is expected at 25 % reaching 40-77 ppb[2,7]. Numerous experimental studies have demonstrated the effect on cereal grain yield by elevated temperature, [CO_2] and [O_3] as single-factors; increasing production by [CO_2][8] and decreasing production by temperature[9] and [O_3][10]. Less studies have reported the effect on grain yield by the combination of climatic factors[11–14]. In studies of elevated temperature and [CO_2] combined, grain yield was found to decrease by 14-53 %[15–17]. The decrease in cereal grain yield with a global temperature increase of > +3 ˚C is critical in the context of maintaining a sufficient primary production, which can meet the needs of a growing world population with rise in living standards.

Grain protein concentration (GPC) has been reported to increase in response to abiotic stress such as heat, drought and elevated [O_3][14,18–20], while GPC was decreased by elevated [CO_2][21]. Timing of the climate effect in plant development was further found to influenced the response in

GPC[3,22]. Grain protein is decisive for several end-uses, however, the effects on GPC by combined climatic factors is little studied even though factors of climate change will appear concerted[3,23].

The fourth major cereal of the world is barley (*Hordeum vulgare* L), which in temperate climates is cultivated predominantly as a spring crop for feed to livestock and malt for use in brewing and distilling industries. Barley has though, within the recent years, achieved increased attention for human consumption due to its high nutritional value and potential health benefits[24]. The diverse uses of barley grains cause different demands to the grain composition. Generally, high protein content is preferable in barley for feed, whereas a low protein grain and high starch content is preferred for malting purposes. Climate change alterations in protein content can in the industrialized countries in the temperate zone also have substantial marked implications with economic and social consequences.

In the context of climate change with projected decreased grain yields[4], the grain protein harvested (GPH) is important for product quality and secured primary production. Few studies have focused on the impact of climate change on cereal grain protein determining for the quality and even fewer in the context of GPH in barley. The objective of this study was to examine climate change effects to an array of accessions. Here we present the effect on grain yield and GPC under the combination of elevated [CO_2] and temperature as under the single-factors elevated [CO_2], temperature and [O_3] on 108 spring barley accessions.

MATERIAL AND METHODS

Plant material. One hundred and eight 2- or 6-rowed primarily Nordic spring barley (*Hordeum vulgare* L.) accessions were included in the study (Table 1). The set included 38 landrace accessions, 25 old cultivars (before 1975), 41 modern cultivars (after 1975) and four breeder-lines. Accessions were supplied by NordGen or Nordic barley breeding companies. For pedigree, breeder institute, and where to order please see S1.

Experimental set up. Five climate treatments were applied in the RERAF phytotron (Risø Environmental Risk Assessment Facility) at the Technical University of Denmark, Roskilde (http://www.eco.kt.dtu.dk/Research/Research_Facilitites/RERAF) to all 108 accessions throughout their full lifecycle. The 108 accessions were a subset of the 138 accessions analysed by Ingvordsen et al.[17] for quantity of production. Within each of the five 24 m^2 chambers (height 3m) in the phytotron, humidity, temperature and gasses were controlled as well as continuously monitored. The five applied treatments can be seen in Table 2. They included (1) ambient (control) mimicking present south Scandinavian summer of 19/12 °C (day/night), [CO_2] constantly at 385 ppm (though without added [O_3]), (2) [CO_2] constantly at 700 ppm, (3) temperature elevated +5 °C (day and night), (4) elevated temperature and [CO_2] combined at the level of the single-factor treatments and (5) [O_3] constantly at 100-150 ppb (day and night). The climatic factors were mimicking levels predicted ultimo 21st century, if greenhouse gasses are not substantially reduced (SCRES scenario A1FI, IPCC, 2007). The CO_2 was supplied by Air Liquide A/S Denmark and O_3 by UV Pro 550A generators (Crystal air products & services, Canada). Further details on RERAF are given by Frenck *et al.*[13] and Ingvordsen *et al.*[17]. Eight plants of each accession were grown in 11 L pots with 4 kg of sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S, Denmark), where 10 g of NPK fertilizer (21-3-10, Yara) was applied at sowing. Water was applied within one hour after the light was turned on by a surface dripping system delivering 4.4 L m^{-2} day^{-1} in all treatments. To compensate for the drainage of the pot setup as well as root distribution and water loss, water was applied above the average precipitation of Southern Scandinavia (236 mm; DMI, 2014). Watering was stepwise reduced from Zadoks growth stage (ZGS) 90 and ended at ZGS 99[27]. Light was supplied by 28 high-pressure mercury (1000 W or 400 W) and 14 halogen (250 W) lamps in each chamber. The daily light cycle was 16/8 h (day/night) and PAR (parabolic aluminized reflector) averaged at approximately 400 mol photons m^{-2} s^{-1} at canopy height (ca. 1 m). To avoid possible chamber specific effects the treatments with its corresponding batch of plants were rotated between the chambers on a weekly basis. In practice all plants were exposed to ambient conditions for approximately 2 hours during the time of rotation and the time necessary for the new chamber to reach the different treatment values.

Grain yield. Plants were harvested individually and after drying in constant temperature for a minimum of three weeks, they were threshed and grain weight measured. After threshing grains were stored at 7 °C. Number of grains was obtained by dividing with the weight of an enumerated sub-sample.

Protein. Total nitrogen (N)-analyses and following calculation of crude protein were performed by YARA (Yara Analytical Services, Pocklington, England) on 5-10 g of grain material via the Dumas Combustion method on a LECO CNS TRUMAC. Crude protein was achieved on 17 accessions (stated in S1) in each of the five treatments, and used to predict protein concentration in the remaining accessions.

NIR measurements. Spectral reflectance of whole kernels from all accessions was obtained using a QFA-Flex 600F FT-NIR instrument (Q-interline, Tølløse, Denmark). 1.5-7 g of the kernels were placed in IR transparent glass vials (height 6 cm, diameter 2.6 cm) and measured using a rotating sample device. The sample was rotated at three rounds per minute. The measuring sample window at the rotating sample device had a diameter of 6 mm, which provides an analysis surface of approximately 510 mm^2. Spectra were collected at every 2 nm in the NIR region from 1100 to 2498 nm. One spectrum was obtained for each sample as an average of 64 sub-scans. The spectra were reported as log (1/R).

Statistics. Principal component analysis (PCA) was performed on raw data as an explorative data analysis to obtain a first overview of the data and to identify obvious outliers and delineate classes. Hotelling's T-square versus residual plots was used to detect outliers. Partial least squares regression (PLSR) models were developed on raw scatter corrected by the Savitsky-Golay first derivative[28] averaging over 7 points and a second order polynomial, and multiplicative signal corrected (MSC)[29]. Root mean square error of cross validation (RMSECV) plotted against the number of PLSR latent variables for each pre-processing method was used to select the optimum pre-processing method and the optimum number of latent variables in the PLSR model. The optimum number of latent variables was chosen as the first local minimum in the smooth declining RMSECV curve or the point, where this curve flattened. Random cross validation with 10 segments and 10 iterations was used.

The performance of the PLSR model to predict protein were evaluated using the root mean square error of prediction (RMSEP), standard error of performance (SEP) and bias. Initially the obtained model was developed on 17 accessions per treatment and used to predict protein concentration in the remaining accessions.

All analysis were carried out using MATLAB version 7.9.0 (R2009b) (The Mathworks, Inc., Natick, MA, USA) along with the PLS toolbox version 7.5.1 (Eigenvector Research, Inc., Manson, WA, USA).

Following statistical analysis was carried out in R version 2.15.3[30], and SigmaPlot version 11.0, from Systat Software, Inc., San Jose California USA, (www.sigmaplot.com) was used for illustration.

RESULTS AND DISCUSSION

Quality of applied treatments. Atmospheric conditions of temperature and relative humidity were during cultivation in rather good agreement with set points programmed in the RERAF phytotron. With regard to experimental levels of [CO_2] the difference between the treatment of ambient and elevated [CO_2] was app. + 240 ppm and on average 75 ppm lower than expected (Table 2). The increased [CO_2] in treatments with ambient levels of [CO_2] is most probably due to that CO_2 cannot technically be removed, and the large amount of plants seemed to have produced considerable quantities of CO_2 during respiration.

Treatment effects on grain yield. The effects of the single climatic factors on overall grain yield of the 108 accessions were reported as a subset of 138 accessions accounted for in Ingvordsen et al.[17]. Grain yield was in agreement with previous studies, reporting increasing grain yield at elevated [CO_2][31] and decreasing grain yield at elevated [O_3][32] and elevated temperature[33] as under the two-factor treatment of elevated temperature and [CO_2] combined[15].

The vast variation in response to the applied climatic treatments will be reported as GPH in the sections 'Treatment effects on GPH' and 'Grain protein in the 108 accessions'.

NIR and prediction of GPC. A PLSR model based on NIR measurements and chemical measurement of N with subsequent calculation of GPC was developed and used to predict GPC in the remaining accessions. Spectra for all included accessions showed sufficient variance and clear peaks for further analysis (Fig. 1). A good calibration model using 8 latent variables on MSC pre-processed NIR spectra showed an R^2=0.8 with an RMSECV=1.34. Based on this calibration model the protein concentration was predicted in the remaining accessions (Fig. 2).

Treatment effects on GPC. The strongest effect on the overall GPC causing a 29 % increase was produced by elevated temperature as single-factor (Table 3). Several studies have reported increasing GPC from elevated temperature >35 °C[18,34] or around anthesis [35,36]. In the present study, a constantly elevated temperature of +5 °C was also found to increase GPC. Högy *et al.*[23] found no change in GPC from a 2 °C increase in soil temperature, but decreased concentrations of total non-structural carbohydrates, starch, fructose and raffinose. The increase in GPC appears promising in terms of securing sufficient protein production under future climate conditions, however, in a future climate [CO_2] is expected to increase concerted with temperature.

Under elevated [CO_2] the GPC decreased overall 5 % (Table 3). This was less than the 15 % decrease found in a meta-analysis of barley with no significant difference between FACE, open-top chambers and enclosure studies or if rooted in pots or field [37]. The less decrease induced by elevated [CO_2] found in the present study might be due to the plant material tested, as elevated levels of [CO_2] were in agreement (590-700). The material used in the meta-analysis presumably covered four barley cultivars (Thule, Alexis, Jo1621 and Atem)[38–41], whereas the present study included 108 accessions (Table 1 and S1).

Elevated [O_3] was found to increase overall GPC with 6 % (Table 3). Studies in wheat, which has been reported more sensitive to [O_3] than barley[42], have found GPC of wheat to increase overall 7 % with averaged [O_3] of 58 ppb and exposure between 7-12 hours per day[32]. The study by Feng et al.[32] also reported 71 ppb [O_3] to cause further increased protein concentration. In the present study [O_3] averaged 121 ppb on a 24 hours basis. The similar increase in GPC from the double concentration and exposure-time to [O_3] may suggest that barley is not very sensitive to O_3 or that barley has a different responds pattern to ozone than wheat.

The 29 % increased GPC under elevated temperature was modified to 8 % under the simultaneous exposure to elevated temperature and [CO_2] in the two-factor treatment (Table 3). This result strongly points to the risk of misinterpretation of the combined effects, when deduced from single-factor treatments. The combined effect of elevated temperature and elevated [CO_2] on GPC was not found to be additive - an important point when considering the future effects of climate change, where temperature, [CO_2] and [O_3] are predicted to increase concerted.

Treatment effects on grain protein pr. grain. Considering the quantity of grain protein in relation to the weight of a single grain (Table 3), the picture changed from increase to decrease under elevated [O_3] and the two-factor treatment compared to response in GPC. Under elevated [CO_2] the GPC and protein per grain decreased similarly, 5 % (Table 3). The 29 % increase in GPC under elevated temperature was substantially lowered to only 7 % when given on a pr. single grain weight basis. The decreases reflected the diverse seed weights in the different treatments, where only the treatment with elevated [CO_2], had more or less the same seed weight as found under ambient conditions (data not shown). However, a suggested inhibition of the assimilation of nitrate into e.g. proteins under elevated [CO_2][43] could have engaged in the maintained and not increased grain weight by elevated [CO_2]. Further, the increase in GPH was found to be smaller than the increase in grain yield as previously reported as indication on inhibition of the assimilation of nitrate under elevated [CO_2][14].

Treatment effects on GPH. The increased GPC in the two-factor treatment that could potentially increase protein production under future climate conditions vanished, when the treatment effect on actual harvested protein was considered (Table 3). Even though the GPC increased 8 % compared to ambient, the GPH was found decreased by 23 % due to the decreased grain yield of 28 % in an atmosphere of elevated temperature plus [CO_2]. The treatments effects on grain yield converted the potential increase in GPH into an overall reduction, as was also seen in the single-factor treatments with elevated temperature and [O_3]. In the single-factor treatment with elevated [CO_2] the opposite was observed, as the decreased GPC was compensated for by the higher yield, and the resulting GPH was increased compared to ambient (Table 3).

Since the findings of the present study are based on 108 accessions the overall effects reported are considered robust with regard to barley, and the characteristics identified might be considered of value in future breeding. Responsiveness to the elevated [CO_2] has been suggested as a breeding target to increase grain yield under future climate conditions[17,31,44]. In the present study we found that the GPC under elevated [CO_2] was decreased, though relatively little in comparison to the increased grain yield, suggesting that a substantial increase in GPH could be envisaged from improved CO_2-responsiveness. Harvested grain protein was found increased (13 %) under elevated [CO_2] - not from increased protein pr. grain but from increased production of grains (Table 3)[17,45]. Application of additional nitrogen-fertilizer could potential ameliorate the loss of protein in the grain under elevated [CO_2], however, Bloom *et al.*[46] reported an insignificant effect on GPC in wheat leaves under elevated [CO_2] due to inhibited nitrate assimilation. The suggested inhibition of protein accumulation by elevated [CO_2] requests better understanding of ammonium and nitrate use by crops under climate change conditions, an area that has received little attention[47].

Grain protein in the 108 accessions. Among the 108 accessions, some differed to a greater or lesser extent from the overall responses to the treatments, suggesting great diversity that could be exploited in breeding programs. No significant difference in response to the climate treatments were observed between the group of landrace and the group of cultivars (Fig. 3 and 4).

Considering the expected lower grain yield under future climate conditions harvesting as much protein as possible is likely preferable. Under the two-factor treatment the 108 accessions decreased in average 23 % in GPH relative to ambient, however, the individual accessions spanned from -60% to 30 % GPH (Fig. 3). Two landraces (Kushteki and Moscou) and a 2-rowed Danish feed barley cultivar (Jacinta) increased 30-33 % in GPH. All three accessions ranked in top ten for grain yield of the studied 108 accessions, whereas only the feed barley ranked in the top (placed 2) in GPC in the two-factor treatment. Another four accessions, two modern cultivars (Sebastian and Brage), a Finish landrace (Luusua) and a breeder-line (Bor 05135) increased 13-16 % in GPH under elevated temperature and [CO_2] in combination. Of these four accessions, only the landrace demonstrated high rank (8) with regard to GPC under the two-factor treatment of all 108 accessions, and the Danish cultivar (Brage) demonstrated high grain yield. The last two accessions, a Norwegian 6-

rowed cultivar and the Finish 2-rowed breeder-line demonstrated top-medium rank for GPC and grain yield, where they ranked 52 and 21 in GPC and 22 and 14 in grain yield. When only considering the performance in the two-factor treatment, all seven accessions rank in top 15 of the 108 accessions in high GPH. That increased GPH was identified in landraces, cultivars and a breeder-line as in 2- and 6-rowed suggest that beneficial genes for developing cultivars with high GPC and grain yield are available from many sources.

Under elevated [CO_2], three accessions increased over 80 % in GPH. The accessions were two 2-rowed old Swedish accessions (Arla and Pallas) and the 2-rowed Danish feed cultivar that also demonstrated increased grain yield under the two-factor treatment (Jacinta). In the pedigrees of both Jacinta and Arla the accession Bavaria (NGB6945) can be found[48]. In addition both Jacinta and Arla showed high CO_2-responsiveness, both placed in top five of accessions increasing most in grain yield under the treatment of elevated [CO_2]. All three accessions had high grain yields under elevated [CO_2], whereas under ambient conditions the old Swedish cultivars ranked low (81 and 106) in GPC and grain yield (79 and 96).

Elevated temperature increased overall GPC the most and was only found decreased in eight accessions being landraces and old cultivars. Overall grain protein harvested was decreased by 42 % but three accessions showed increased GPH under elevated temperature, all 2-rowed and cultivars; an old Swedish (Mari), an old Danish (Odin) and a modern Danish (Sebastian).

One can speculate if the ability of Jacinta and Sebastian to produce high GPH in the two-factor treatment was related to their suggested improved ability to secure high GPH in either of the single treatments of elevated [CO_2] or elevated temperature – or reverse; the performance in either of the single-factor treatments contributed to the performance under the combined treatment. However, the results from single-factor treatments were overall not found additive for the two-factor treatment, and of the mentioned accessions only two were found in top for GPH under either of the single-factor treatments and the two-factor treatment. Considering more accessions than the top three to five best ones, though revealed broader overlap of accessions producing high GPH under the two-factor treatment and either of both of the single-factors suggesting that high performance under a single-factor treatment can be beneficial in the two-factor treatment.

Under elevated [O_3], several accessions decreased less than the averaged 11 %, and 14 accessions increased > 11 % with regard to GHP. Cultivar variation to [O_3] have previously been reported in grain yield of soybean by Betzelberger et al.[49] (with [O_3] applied eight hours a day at 40-150 ppb) and for the set of present accessions in Ingvordsen *et al.*[17]. Two old cultivars (Pallas and Juli) and an early modern cultivar (1978; Agneta) showed highest increased GHP under elevated [O_3] and Agneta also ranking 1 with regard to GPH under the [O_3] treatment of all 108 accessions.

The massive variation in protein responds to the applied climate treatments, emphasize that the phenotypic differences should be exploited in breeding programs for abiotic stress tolerance. Likely, a cascade of different genes encodes the different responses. Here, mining the genome with GWAS (Genome Wide Association Studies) could help identifying some of the underlying genes, and the link between these DNA markers and phenotypes could facilitate the breeding process. Additionally, the identification of suitable genetic resources should be performed under treatments of combined climatic factors, since the effects from the single-factors were found rarely to be additive. The overall decreased GPH in the most realistic climate treatment, where [CO_2] and temperature were elevated simultaneously, emphasizes the need to explore and exploit genotypes to secure plant protein production under future climate conditions.

ABBREVATION USED

GPC: grain protein concentration; GPH: grain protein harvested: MSC: multiplicative signal corrected; NIR: Near infrared radiation; PLSR: partial least squares regression; RERAF: Risø Environmental RiskAssessment Facility; RMSECV: root mean square error of cross validation; RMSEP: root mean square error of prediction; SEP: standard error of performance

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ASSOCIATED CONTENT

Supporting Information

Table S1 The 108 spring barley accessions included in present study with genebank number, accession type, row type, origin country/breeding country, collecting date/release year, location/breeder institute, pedigree and marking of the 17 individual included in the chemical analysis.

This material is available in print in the dissertation and digitally on the attached CD-rom.

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Notes

The authors declare no competing financial interest

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Table 1. Overview of the accessions included. Old cultivars before 1975; modern cultivars after 1975.

	Landraces		Old cultivars		Modern cultivars[a]	
	2 rowed	6 rowed	2 rowed	6 rowed	2 rowed	6 rowed
Denmark	1	2	6	1	23	
Sweden	1	1	7	2	2	1
Finland[b]	2	5	1	4	2	6
Norway	1		1	3		4
Europe[c]	5	7			7	
non-Europe	1	4				
unknown	1	5				

[a]including breeder-lines

[b]two landraces segregated either as 2 or 6 rowed and has not been included

[c]not including Scandinavian but Faroe Islands

Table 2. Experimental levels of manipulated climatic factors of applied treatments. Set points were; temperature (tmp): 19/12 °C (day/night) or 24/17 °C; [CO_2] (CO_2): 385 ppm or 700 ppm; [O_3] (O_3): 100-150 ppb; relative humidity 55/70 % day/night.

	tmp day/night	[CO_2] (constant)	[O_3] (constant)	humidity day/night
ambient	18.9±1.2/11.8±0.8	448.5±81.1	1.40±1.4	55.7±2.5/69.9±1.5
+CO_2	19.0±1.2/12.5±2.1	684.7±41.1	0.98±1.7	55.3±5.1/69.4±5.9
+tmp	23.9±1.4/16.8±0.8	448.4±74.4	1.90±1.2	55.9±2.8/69.8±1.6
+tmp & CO_2	23.8±1.3/16.9±0.9	688.3±38.2	1.50±1.4	56.0±2.9/69.8±1.8
+O_3	18.9±1.2/11.9±1.0	443.1±67.5	121.1±32.8	55.7±2.4/69.8±1.7

Table 3. Overall averaged parameters for the 108 barley accessions cultivated under future levels of carbon dioxide ($+CO_2$), ozone ($+O_3$), temperature (+tmp) and under the two-factor treatment (+tmp & CO_2) as well as under ambient (amb). * specifies significant difference from the ambient treatment determined by t-test. Grain yield per plant and grain number per plant are from Ingvordsen *et al*.[17]

	amb	+tmp & CO_2	+ CO_2	$+O_3$	+tmp
Grain yield per plant (g)	6.85±1.29	4.92±1.18***	8.02±1.94***	5.82±1.38***	3.08±1.13***
% different from ambient		*-28.12*	*17.10*	*-15.10*	*-54.98*
Grain number per plant (#)	128.02±31.2	100.01±25.3***	149.93±17.1***	122.21±31.9	68.77±24.1***
% different from ambient		*-21.88*	*17.11*	*-4.54*	*-46.54*
Grain protein concentration (%)	13.97±1.82	15.06±1.97***	13.33±1.91*	14.76±1.96**	18.03±2.18***
% different from ambient		*7.86*	*-4.85*	*5.68*	*29.11*
Grain protein/grain (mg)	7.62±1.42	7.49±1.32	7.24±1.66	7.09±1.35**	8.14±1.62*
% different from ambient		*-1.63*	*-4.87*	*-6.84*	*6.82*
Grain protein harvested per plant (g)	0.95±0.20	0.74±0.19***	1.07±0.31**	0.85±0.18***	0.55±0.20***
% different from ambient		*-22.53*	*12.46*	*-11.19*	*-42.26*

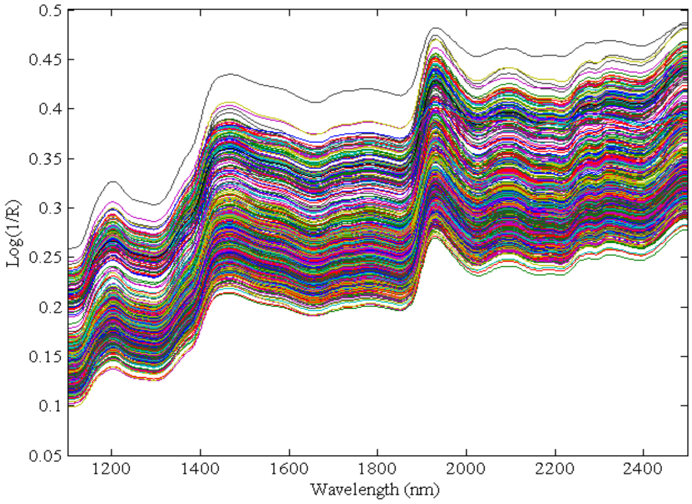

Figure 1. Raw NIR spectra (1100 to 2498 nm) of all accessions.

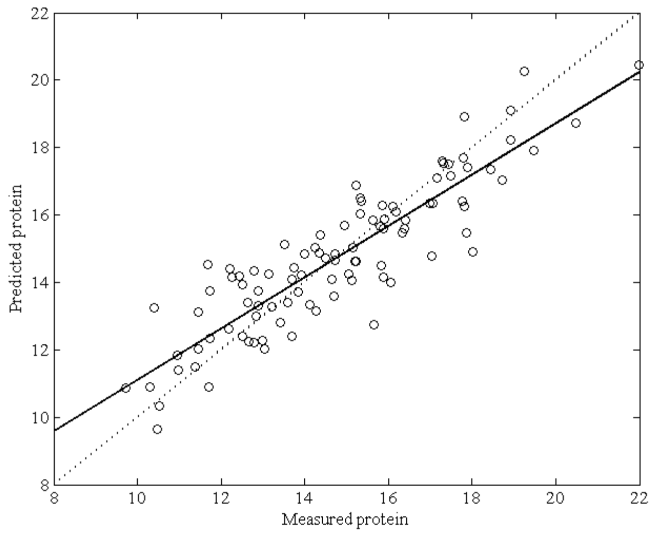

Figure 2. Concentration of protein predicted using PLSR model vs. measured protein concentration. Full line indicate best fit with R^2=0.8 and RMSECV=1.3392. Dotted line has R^2=1

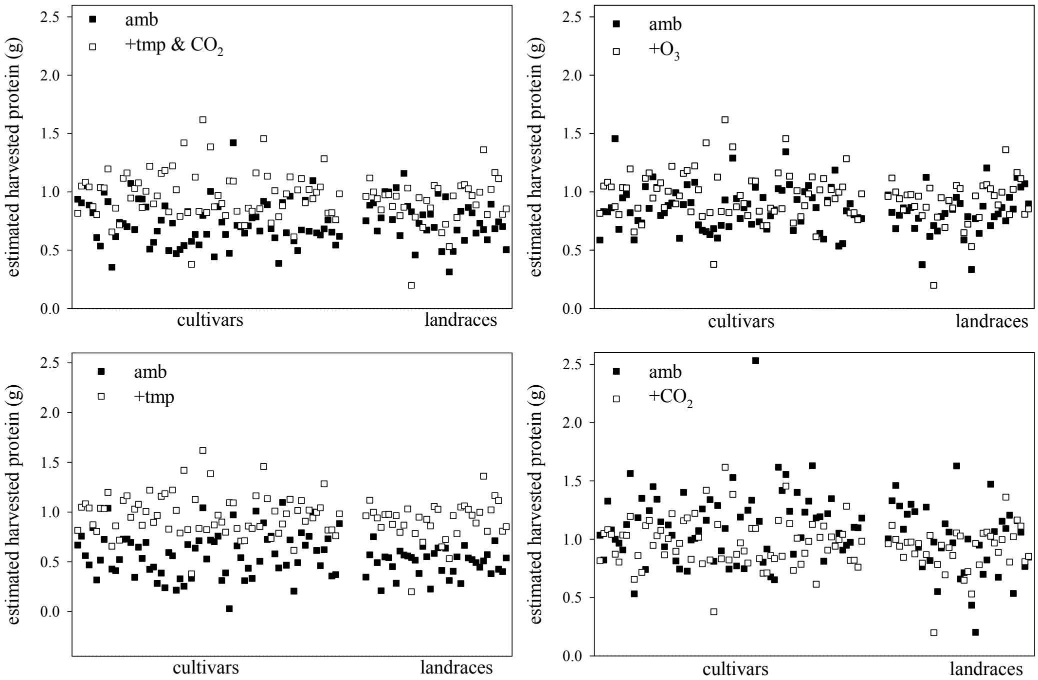

Figure 3. Grain protein harvested (g/plant) of the 108 accessions in the five climate treatments (open) and ambient (closed). The order of accessions, left to right, is as in S1.

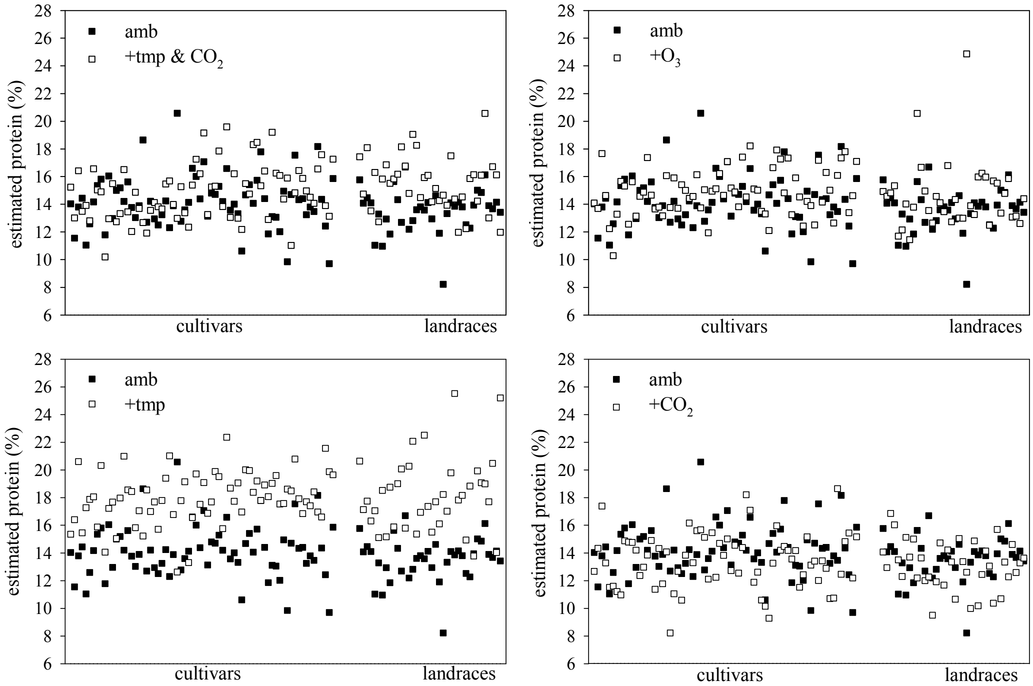

Figure 4. Grain protein concentration (%) of the 108 accessions in the five climate treatments (open) and ambient (closed). The order of accessions, left to right, is as in S1.

Supplementary material

Manuscript 3

SUPPORTING INFORMATION

Table S1. Information on accessions included.

'*' indiates the accessions that were included in N-analysis performed by YARA (Yara Analytical Services, Pocklington, England)

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Bor05135	Bor05135		BL	Finland	2		Boreal Plant Breeding
Bor09801	Bor09801		BL	Finland	6		Boreal Plant Breeding
NOS 15251-52	NOS 15251-52		BL	Denmark	2		Nordic Seed A/S Plant Breeding
NOS 17009-53	NOS 17009-53		BL	Denmark	2	expected 2014	Nordic Seed A/S Plant Breeding
Agneta	Agneta	NGB1508	CV	Sweden	6	1978	NordGen
Alabama	Alabama		CV	Germany	2	1999	BAR-OF project (ICROFS,Denmark)
Alf	Alf*	NGB4707	CV	Denmark	2	1978	NordGen
Alliot	Alliot*	NGB16757	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Amalika	Amalika		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Anakin	Anakin*		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Anita	Anita*	NGB15250	CV	Norway	6	1962	NordGen
Arla	Arla	NGB2681	CV	Sweden	2	1962	NordGen
Åsa	Åsa	NGB4640	CV	Sweden	6	1949	NordGen
Birka	Birka	NGB4712	CV	Sweden	2	1981	NordGen
Brage	Brage*		CV	Norway	6	2010	Graminor Plant Breeding
Brazil	Brazil		CV	France	2	2000	BAR-OF project (ICROFS,Denmark)
Brio	Brio*	NGB9327	CV	Sweden	6	1924	NordGen
Calisi	Calisi		CV	Denmark	2	2013	Nordic Seed A/S Plant Breeding
Caruso	Caruso	NGB15059	CV	Denmark	2	1991	NordGen
Chevallier Tystofte	Chevallier Tystofte	NGB9443	CV	Denmark	2	1883	NordGen
Cicero	Cicero	NGB16756	CV	Denmark	2	1999	BAR-OF project (ICROFS,Denmark)
Culma	Culma		CV	Belgium	2	1997	BAR-OF project (ICROFS,Denmark)
Danpro	Danpro	NGB9659	CV	Denmark	2	1969	NordGen
Danuta	Danuta		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Denso Abed	Denso Abed	NGB8826	CV	Denmark	2	before 1965	NordGen
Drost Pajbjerg*	Drost Pajbjerg*	NGB6281	CV	Denmark	2	1951	NordGen
Edvin*	Edvin*		CV	Finland	6	2008	Boreal Plant Breeding
Elmeri	Elmeri		CV	Finland	6	2009	Boreal Plant Breeding
Etu	Etu	NGB2679	CV	Finland	6	1970	NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Eva	Eva	NGB1502	CV	Sweden	2	1973	NordGen
Fabel Sejet	Fabel Sejet		CV	Denmark	2	2001	BAR-OF project (ICROFS,Denmark)
Fero	Fero	NGB8231	CV	Denmark	2	1943	NordGen
Fræg	Fræg	NGB8860	CV	Norway	6	1948	NordGen
Freja	Freja	NGB1485	CV	Sweden	2	1941	NordGen
Galant Carlberg	Galant Carlberg	NGB6308	CV	Denmark	2	1985	NordGen
Gaute	Gaute	NGB16732	CV	Norway	6	2000	Graminor Plant Breeding
Hannchen	Hannchen	NGB4617	CV	Sweden	2	1902	NordGen
Harbinger	Harbinger		CV	Finland	2	2009	Boreal Plant Breeding
Harriot	Harriot		CV	Germany	2	2001	BAR-OF project (ICROFS,Denmark)
Helium	Helium		CV	Denmark	2	2001	Nordic Seed A/S Plant Breeding
Hydrogen	Hydrogen	NGB20346	CV	Denmark	2	2000	Nordic Seed A/S Plant Breeding
Jacinta	Jacinta	NGB16665	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Jadar II	Jadar II	NGB457	CV	Norway	6	1947	NordGen
Juli Abed	Juli Abed	NGB4585	CV	Denmark	6	1909	NordGen
Justus	Justus		CV	Finland	6	2013	Boreal Plant Breeding
Karri	Karri	NGB287	CV	Finland	2	1967	NordGen
Landora	Landora		CV	Germany	2	2000	BAR-OF project (ICROFS,Denmark)
Laurikka	Laurikka		CV	Denmark	2	2012	Nordic Seed A/S Plant Breeding
Lavrans	Lavrans	NGB16727	CV	Norway	6	1999	Graminor Plant Breeding
Linus	Linus	NGB13482	CV	Sweden	2	1997	NordGen
Lysimax	Lysimax	NGB15055	CV	Denmark	2	1994	NordGen
Mari	Mari*	NGB1491	CV	Sweden	2	1960	NordGen
Møyjar	Møyjar	NGB2106	CV	Norway	2	1969	NordGen
Nord	Nord	NGB9942	CV	Finland	6	1988	NordGen
Odin	Odin	NGB16755	CV	Denmark	2	1981	NordGen
Orthega	Orthega		CV	Germany	2	1997	BAR-OF project (ICROFS,Denmark)
Paavo	Paavo	NGB13661	CV	Finland	6	1959	NordGen
Pallas	Pallas		CV	Sweden	2	1970	BAR-OF project (ICROFS,Denmark)
Pinocchio	Pinocchio		CV	Denmark	2	2011	Sejet Plant Breeding I/S
Pirkka	Pirkka	NGB292	CV	Finland	6	1952	NordGen
Prestige	Prestige*	NGB16750	CV	France	2	2000	The Danish AgriFish Agency, Tystofte
Prominant	Prominant	NGB15066	CV	Denmark	2	1999	NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Sebastian	Sebastian*		CV	Denmark	2	2002	NordGen
Severi	Severi		CV	Finland	6	2013	Boreal Plant Breeding
Simba	Simba		CV	Denmark	2	2002	NordGen
Tammi	Tammi	NGB6925	CV	Finland	6	1937	NordGen
Tore	Tore*	NGB6605	CV	Norway	6	1986	NordGen
Tron Sejet	Tron Sejet	NGB9655	CV	Denmark	2	1978	NordGen
Visir	Visir	NGB1496	CV	Sweden	2	1971	NordGen
Zita	Zita	NGB7236	CV	Denmark	2	1974	NordGen
Anita Högsby-korn	Anita Högsby-korn	NGB263	L	Sweden	2		NordGen
Bjørne	Bjørne*	NGB9326	L		6		NordGen
Bryssel	Bryssel KVL 28	NGB8222	L	Belgium	6		NordGen
Cluj	Cluj KVL 100	NGB9355	L	Romania	6		NordGen
Gardez Pandshir	Gardez Pandshir K.173	NGB4669	L	Afghanistan	6	194811--	NordGen
Griechische	Griechische KVL 56*	NGB9333	L	Greece	6		NordGen
Hannuksela	Hannuksela	NGB325	L	Finland	6		NordGen
Junkkari	Junkkari	NGB307	L	Finland	6		NordGen
Kilpau	Kilpau ME0201	NGB1159	L	Finland	6	19810819	NordGen
Königsberg	Königsberg KVL 18*	NGB9310	L		6		NordGen
Kushteki	Kushteki K.77*	NGB6288	L	Afghanistan	6	1948----	NordGen
Langaks	Langaks	NGB6300	L	Faroe Islands	6	196108--	NordGen
Lantkorn från Jämtland	Lantkorn från Jämtland	NGB6927	L	Sweden	6		NordGen
Laukko	Laukko	NGB16881	L	Finland	2		NordGen
Ljubljana KVL 15	Ljubljana KVL 15	NGB9308	L	Croatia	6		NordGen
Luusua	Luusua EH0401	NGB792	L	Finland	6	19790907	NordGen
Lynderupgaard	Lynderupgaard	NGB9529	L	Denmark	6	195205--	NordGen
Magdeburg	Magdeburg KVL 358	NGB9485	L	Germany	2		NordGen
Manschurei	Manschurei	NGB9624	L		6		NordGen
Metz	Metz KVL 124	NGB9373	L	France	6		NordGen
Montpellier	Montpellier KVL 209	NGB9410	L	France	2		NordGen
Moscou	Moscou KVL 353*	NGB9482	L		2		NordGen
Näckte von Nepal	Näckte von Nepal	NGB9305	L	Nepal	6		NordGen
Nordslesvigsk Kæmpe	Nordslesvigsk Kæmpe	NGB9339	L	Denmark	6	1890	NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Nue Grosse	Nue Grosse	NGB9436	L	Denmark	2		NordGen
Oppdal	Oppdal	NGB13670	L	Norway	6		NordGen
Osiris	Osiris J-1277	NGB9639	L		6		NordGen
Pavia	Pavia KVL 386	NGB9501	L	Italy	2		NordGen
Peruvian	Peruvian	NGB8880	L	Peru	6		NordGen
Rauto	Rauto	NGB265	L	Finland	2;6		NordGen
Rehakka	Rehakka-65	NGB314	L	Finland	2		NordGen
Sarkalahti	Sarkalahti ME0103	NGB27	L	Finland	6	19800810	NordGen
Smolensk	Smolensk KVL 741	NGB9623	L	Russian Federation	2		NordGen
Stjernebyg fra Færøerne	Stjernebyg fra Færøerne	NGB4701	L	Faroe Islands	6	196908--	NordGen
Szeged	Szeged KVL 347	NGB9478	L	Hungary	2		NordGen
Tartu	Tartu KVL 349	NGB9479	L	Estonia	2		NordGen

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Vilmorin	Vilmorin KVL 126	NGB9375	L		6		NordGen
Ylenjoki	Ylenjoki AP0301	NGB4413	L	Finland	2;6	19830825	NordGen

Accession name	Location / Breeder institute	Culton pedigree
Bor05135	Boreal Plant Breeding	
Bor09801	Boreal Plant Breeding	
NOS 15251-52	Nordic Seed A/S Plant Breeding	
NOS 17009-53	Nordic Seed A/S Plant Breeding	Iron x Keops
Agneta	Sveriges Utsadesforening Svalöf	(Åsa x Frisia) ^2 x (Edda II ^4 x Monte Cristo)
Alabama	KWS SAAT AG	(Ml-i x 2.51784) x Krona
Alf*	Nordic Seed A/S Plant Breeding	Mutant in Bomi
Alliot*	Nordic Seed A/S Plant Breeding	Waggon*Imidis
Amalika	Nordic Seed A/S Plant Breeding	NOS 1184-07 x Quench
Anakin*	Sejet Plant Breeding I/S	Tumbler x Respons
Anita*	Norwegian Agrcultural University	HOL-44 x Arve; / (Asplund x Ds295) x Varde
Arla	Weibullsholm Plant Breeding Institute	(Maja x ((Hanna x Svanhals) x Opal)) x Tammi
Åsa	Sveriges Utsadesforening Svalöf	Dore x Vega
Birka	Weibullsholm Plant Breeding Institute	W 82-68 x W 17-68

Accession name	Location / Breeder institute	Culton pedigree
Brage*	Graminor Plant Breeding	Lavrans/NK91650
Brazil	KWS SAAT AG	Trebon x Cooper
Brio*	Sveriges Utsadesforening Svalöf	Selection from 6-row barley from Skåne
Calisi	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Caruso	Carlsberg	(Rupal X Terrax) X Grit
Chevallier Tystofte		
Cicero	Sejet Plant Breeding I/S	Chalice x SJ 933275
Culma	Limagrain	590907 x Hart
Danpro	Carlsberg	Proctor x Dana
Danuta	NordSaat	90014DH x (Salome x Maresi)
Denso Abed	The Abed Foundation	Rigel
Drost Pajbjerg*	The Pajbjerg Foundation	Maja x Kenia
Edvin*	Boreal Plant Breeding	Verner/Hja 85194
Elmeri	Boreal Plant Breeding	Thule/Verner
Etu	Agricultural Research Centre	Bonus M x Varde
Eva	Sveriges Utsadesforening Svalöf	Birgitta x Mari
Fabel Sejet	Sejet Plant Breeding I/S	Newgrange x SJ 2256
Fero	Øtofte	Selection from Kenia
Fræg	(SFL)Norwegian Government Res.Station Holt	Asplund x Maskin
Freja	Sveriges Utsadesforening Svalöf	Seger x Opal
Galant Carlberg	Carlsberg	Mutation in Triumph
Gaute	Graminor Plant Breeding	SvN82114/Vo13647-77
Hannchen	Sveriges Utsadesforening Svalöf	Line selection from Hanna
Harbinger	Boreal Plant Breeding	
Harriot	Nordsaat	(GS 1635 x Nordus) x Annabell
Helium	Nordic Seed A/S Plant Breeding	Meltan x Delibes
Hydrogen	Nordic Seed A/S Plant Breeding	(Alis x Digger) x Derkado
Jacinta	Nordic Seed A/S Plant Breeding	(Alexis x Meltan) x Canut
Jadar II	(SFL)Norwegian Government Res.Station Forus	Jadar x Asplund
Juli Abed	The Abed Foundation	Collected material
Justus	Boreal Plant Breeding	
Karri	Hankkija Plant Breeding Station	Carlsberg x Rigel
Landora	Saatzucht Hadmersleben GmbH	Cross breeding (Hadm86508-91 x Hadm46544-88)
Laurikka	Nordic Seed A/S Plant Breeding	(Ceb 7931 x Pompadour) x (577223 x Golf)
Lavrans	Graminor Plant Breeding	Vera//Arve/H82009-1-3

Accession name	Location / Breeder institute	Culton pedigree
Linus	Svalöf Weibull AB	WW 7749 x Ariel
Lysimax	Sejet Plant Breeding I/S	Ca 040223 x Carula
Mari*	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Møyjar	(SFL)Norwegian Government Res.Station Møystad	Domen x Herta
Nord	Lantbrukets Forskningscentrals Växtförädlingsanstalt	Otra x Etu
Odin	Carlsberg	Sv 66433 x ALL.297
Orthega	KWS SAAT AG	Tucson x Quench
Paavo		(Tammi x Gull) x O.A.C. 21
Pallas	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Pinocchio	Sejet Plant Breeding I/S	Quench x SJ Afrodite
Pirkka	Hankkija Plant Breeding Station	TA 04369 x TA 05864
Prestige*	RAGT	AC 00/767/5 x Anakin
Prominant	Sejet Plant Breeding I/S	Caminant x Vintage
Sebastian*	Sejet Plant Breeding I/S	Lux x Viskosa
Severi	Boreal Plant Breeding	
Simba	Sejet Plant Breeding I/S	Otira x Prolog
Tammi	Hankkija Plant Breeding Station	Asplund x Olli
Tore*	Norwegian Agrcultural University /(Graminor)	Lise x Clermont
Tron Sejet	Sejet Plant Breeding I/S	Impala x Nigrate
Visir	Sveriges Utsadesforening Svalöf	Pallas x Long Glumes
Zita	The Pajbjerg Foundation	PF 203/7748 x Vada
Anita Högsby-korn		
Bjørne*		
Bryssel KVL 28		
Cluj KVL 100		
Gardez Pandshir K.173	Gardez Panshir	
Griechische KVL 56*		
Hannuksela		
Junkkari		
Kilpau ME0201	Kilpau, Oulainen	
Königsberg KVL 18*		
Kushteki K.77*	Kushteki	
Langaks	Faroe Islands	
Lantkorn från Jämtland		

Accession name	Location / Breeder institute	Culton pedigree
Laukko		
Ljubljana KVL 15		
Luusua EH0401	Luusua, Kemijärvi	
Lynderupgaard	Lyderupgaard	
Magdeburg KVL 358		
Manschurei		
Metz KVL 124		
Montpellier KVL 209		
Moscou KVL 353*		
Näckte von Nepal		
Nordslesvigsk Kæmpe		
Nue Grosse		
Oppdal		
Osiris J-1277		
Pavia KVL 386		
Peruvian		
Rauto	(SFL)Norwegian Government Res.Station Løken	
Rehakka-65		
Sarkalahti ME0103	Sarkalahti, Luumäki	
Smolensk KVL 741		
Stjernebyg fra Færøerne	Faroe Islands	
Szeged KVL 347		
Tartu KVL 349		
Vilmorin KVL 126		
Ylenjoki AP0301	Jussila, Ylenjoki, Valkeakoski	

Manuscript 4

Effect of an extreme heatwave on 22 spring barley accessions cultivated in future climates. Tendencies in allocation of biomass, temperature priming, CO_2-responsiveness and stability of grain yield

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Keywords *Climate change, climate resilient cultivars, extreme events, extreme temperature, genotype differences*

Abstract

Extreme climate events such as floods, storms and heatwaves are critical constraints to primary production driven by climate change. Heatwaves are predicted to increase in frequency, length and intensity and to affect primary crop production. In Europe summer heatwaves, as experienced in 2003, are projected to increase in the future with severe consequences. In the present study, a 10 day-heatwave of 33/28 °C (day/night) was induced around time of flowering in spring barley. The 22 accessions were grown under ambient conditions similar to south Scandinavian summer and future climate change conditions of constantly +5 °C temperature and 700 ppm atmospheric CO_2 concentration as single-factors and in combination as in SRES scenario A1FI of IPCC. The effects of the climate treatments with and without heatwave exposure were assessed on grain yield, number of ears, biomass, harvest index and climate-stability of the individual accessions. The most likely future climate scenario of combined elevated temperature and [CO_2] with a heatwave exposure lead to 52 % decreased grain yield compared to the ambient scenario without heatwave exposure. However, accessions responded differently to the applied treatments with yield decrease from 83 to 27 % depending on accession. Heatwave exposure changed the allocation of biomass by increasing vegetative aboveground biomass and decreasing grain yield. Accessions with high grain yield under elevated temperature and [CO_2] with heatwave exposure, tended to have high CO_2-responsiveness, but low stability of grain yield over the eights treatments applied. The present study emphasizes the need for assessing the effects of extreme events under climate change conditions and to evaluate and select genetic resources in order to secure the primary production in the changing climate. In addition, original data on responses of numerous cultivars to extreme events is provided to fill the gap in crop modelling of the future.

Introduction

Extreme climate events are predicted to be among the most challenging constraints in the future. Heatwaves, floods, droughts and storms cause acute changes in the environments endangering primary production, human health and material possessions (Fischer and Schär, 2009; Hajat *et al.*, 2010; Collins *et al.*, 2013). Simulation studies together with real data from recent decades have suggested the variability within seasons to be more unfavourable for plant production than the seasonal changes (Reyer *et al.*, 2013; Gourdji *et al.*, 2013). In 2012-2013 Australia experienced what became known as the 'angry summer' where over 100 temperature records were broken

(BoM, 2014). An extreme heatwave caused large scale yield failures in Russia in 2010 (Trenberth and Fasullo, 2012). Europe experienced extreme heatwaves in 2006 and 2003. In 2003 it caused a 21 % decrease in wheat grain yield in France with temperatures elevating up to 6 °C above long-term means and precipitation being less than 50 % of the average (Ciais *et al.*, 2005). In general, summer heatwaves are predicted to become more frequent and severe in the future (Schär *et al.*, 2004; Meehl and Tebaldi, 2004; Fischer and Schär, 2010).

In North Europe, barley - especially spring barley - is the cereal taking up most of the cultivated area (19 %), and grains are used for feed and malt (FAOSTAT 2013). More frequent summer heatwaves together with predicted decrease in precipitation during summer (IPCC, 2007) could decrease grain yield additionally. In Europe, the annual average increase in grain yield of barley and wheat that have been observed since the 'Green revolution' is stagnating (FAOSTAT, 2014). Ray *et al*. (2013) reported that growth rate of yields in wheat, maize, rice and soybean will be insufficient to meet the demand in 2050. Hence, development of climate resilient cultivars could ameliorate the adverse future climate change effects. In the development of climate resilient cultivars, assessing the effects of the most likely and relevant climate changes is essential, despite the evident challenges caused by high natural complexity of timing, frequency and intensity of climatic events and extremes.

The effect of elevated temperature and elevated atmospheric carbon dioxide concentration ([CO_2]) on grain yield has been evaluated as single-factors and in combination under experimental conditions in FACE (free air carbon enrichment) and in enclosure studies as well as in simulation studies (Lawlor and Mitchell, 1991; Conroy *et al.*, 1994; Jablonski *et al.*, 2002; Ainsworth and Long, 2005; Lobell *et al.*, 2011; Clausen *et al.*, 2011; Challinor *et al.*, 2014; Ingvordsen *et al.*, 2014*b*). The numerous studies generally reported decreasing grain yield by elevated temperature and increasing yield from elevated [CO_2]. In combinations, the harmful effect of temperature increase was higher compared to beneficial effect of elevated [CO_2], and therefore, grain yield was generally decreased (Conroy *et al.*, 1994; Long *et al.*, 2006; Ingvordsen *et al.*, 2014*b*). Such reported future decrease in grain yield, however, often ignores the effects of extreme events. Few studies have so far investigated the effect of heatwaves at elevated temperature and [CO_2] conditions on grain yield. One study included heatwave exposure to three wheat cultivars, however, none applied heatwave in addition to elevated temperature and [CO_2] or in their combination.

In the present study, a 10-day heatwave of 33/28 °C (day/night) was induced around time of flowering in spring barley. The 22 barley accessions were grown under ambient condition similar to south Scandinavian summer or future climate change conditions of constantly +5°C elevated temperature and 700 ppm [CO_2] as single-factors and in combination as in SRES scenario A1FI of IPCC (2007). The effects of the climate treatments with and without heatwave exposure were assessed on grain yield, number of ears, biomass, harvest index and climate-stability of accessions.

Material and Methods

Plant material and growing conditions

A diverse set of 22 accessions, comprising landraces, old and new cultivars and breeder-lines were selected due to their performance in previous studies with respect to production and fungal disease resistance under climate change conditions (Ingvordsen *et al.*, 2014*a*,*b*). For details on the 22 accessions see Table 1 and S1. The accessions were supplied by NordGen (the Nordic Genetic Resource Center; http://www.nordgen.org/) and Nordic breeding companies.

Each accession was grown in 11 L pots filled with 4 kg of sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S, Denmark) supplemented with 10 g NPK fertilizer (21-3-10, Yara) at sowing. Twelve seeds were sown and at the seedling stage thinned to eight experimental plants. Throughout the duration of the experiment 4.4 L m^{-2} day^{-1} water was applied at the beginning of the daytime regime by an automated surface dripping system. Excess of water was drained from the pots. The amount of water supplied was sufficient to avoid water limitation during growth under ambient conditions. Light regime mimicked the long days of southern Scandinavia (May-July) with 16 hours of light and 8 hours of dark. Two replicate pots of each accession were placed on wheeled growing, one for continuously exposure to of a basic treatment climate scenario and one for corresponding basic treatment with additional heatwave. At growth day 27, about Zadoks growth stage 15-17 (Zadoks *et al.*, 1974) all plants were treated with Confidor WG 70 (Bayer A/S).

RERAF and applied treatments

The accessions were cultivated in the RERAF (Risø Experimental Risk Assessment Facility) phytotron at Technical University of Denmark, Campus Risø, Roskilde (http://www.eco.kt.dtu.dk/Research/Research_Facilitites/RERAF). RERAF has the advantage of six 24 m^2 (6×4×3) gastight chambers individually programmed and with continuous measurements of the experimental conditions. Lamps can be turned on individually and intensity is in PAR (parabolic aluminized reflector) averaged at approximately 400 mol photons m^{-2} s^{-1} at canopy height (ca. 1 m) as in this study.

In the present experiment, temperature, humidity and [CO_2] were controlled in four basic treatments and four treatments with heatwaves. The basic treatments were detained at constant levels throughout the entire life cycle of the plant. One of the basic treatments, the ambient treatment, mimicked present south Scandinavian summer conditions. From the remaining three basic treatments two were single-factor treatments of either elevated temperature or [CO_2] and in addition to those, one two-factor treatment, where elevated temperature and [CO_2] were combined at levels expected in the Nordic region ultimo 21st century. Set point for the 10 day-heatwave treatment was 33/28 °C (day/night) and it was induced under either elevated [CO_2] or ambient [CO_2]. Experimental settings are shown in Table 2. The possibility to turn lamps on individually was used to start/end each daylight regime with a sunrise/sunset with the duration of one hour each. To deal with potential chamber specific effects the treatments were rotated between chambers once a week. When chamber rotations took place, conditions in all treatments were set to ambient, and the batches of plants were moved to their new chamber and the corresponding treatment applied again. The rotation practice was ended 68 days after sowing to avoid damage, when moving the wheeled tables with developed plants through the chamber doors.

The 10 day-heatwave treatment was initiated for a given accession, when four plants out of eight had reached ZGS 49 in the defined pot. The predefined pot was moved to the heatwave treatments, with or without elevated [CO_2] within one hour after the daytime regime had started (Fig. 1). After the 10 days, the pot was transferred back to its basic treatment within one hour after the daytime regime had started. Throughout the heatwave treatment, controlled watering was applied as in the basic treatments.

Watering was reduced stepwise for each treatment starting at ZGS 90 and watering was ended at ZGS 99. Growth stage was determined on the experimental plants that had not been exposed to heatwave.

Data collection and treatment

Plants were harvested individually and the total number of ears was counted. After drying for a minimum of three weeks ears were thrashed individually and grains (g $plant^{-1}$) and vegetative aboveground biomass (g $plant^{-1}$) were measured. From the measured parameters, total biomass (g plant^{-1}), harvest index (HI; grain yield proportional to vegetative aboveground biomass, %) and grain yield per ears (g ear^{-1}) were calculated. Differences between treatments were calculated as percentage deviation.

Stability measures over the eight treatments were calculated by the static environmental variance (S^2; Roemer, 1917) and the dynamic Wricke's ecovalence (W^2; Wricke, 1962) according to

$$S^2{}_i = \sum (R_{ij} - m_i)^2 / (e - 1) \quad [1]$$

$$W^2{}_i = \sum (R_{ij} - m_i - m_j + m)^2 \quad [2]$$

where R_{ij} is the observed yield of the accession i in the treatment j, m_i is mean yield of the accession across treatments, e is number of environments, m is the average of all m_i termed the grand mean.

Microsoft Excel (2010), R version 2.15.3 (R core Team, 2013) and SigmaPlot version 11.0, from Systat Software, Inc., San Jose California USA, (www.sigmaplot.com) were used for data handling, statistics and figures. T.test determined significance with the code 0.001 = ***, 0.01=** and 0.05 = *.

Results

Experimental values

The levels of the climatic factors applied in RERAF throughout plant cultivation were compatible with set points. Also ambient and elevated experimental levels corresponded in deviation, e.g. relative humidity during the day was set to 55 and spanned 56.43-56.66 over all treatments. Within the treatments of ambient [CO_2] plant respirations might have caused the higher increase averaging 50 ppm than targeted. The highest differences were found within the first hour of day and night regime, hence, indicating the time that the RERAF system used to reach set points. Set points and values are given in Table 2.

Effects of the basic climate treatments

The measured production parameters from the 22 tested accessions are shown in Table 3. In the basic treatments grain yield was found to increase 31 % in the treatment with elevated [CO_2] compared to the ambient, while elevated temperature alone and in combination with elevated [CO_2] decreased overall grain yield by 40 % and 18 %, respectively. Number of ears was found to increase only under the two-factor treatment of elevated temperature and [CO_2].

The vegetative aboveground biomass was decreased by the elevated temperature in the basic treatments; 7.9 %. However, the total vegetative aboveground biomass decreased considerable more, 22.3 % as a consequence of the strong decrease in grain yield. Under elevated [CO_2] the vegetative aboveground biomass and the total aboveground biomass were both found to increase by 30.7 %. In the two-factor treatment the vegetative aboveground biomass was again increased by 19.4 %, whereas the total aboveground biomass increased only by 2.5 % due to the decrease in grain yield.

The accessions on average reached the transfer-stage (ZGS 49) first in the two-factor treatment, specifically 45.5 days after sowing. In the elevated temperature treatment ZGS 49 was reached 48.5 days after sowing and after 50.4 and 50.5 days in the ambient and the elevated [CO_2] treatment, respectively (Fig. 2). Watering was ended at ZGS 99, which was first reached in the two-factor treatment 107 days after sowing. In the treatment of elevated temperature ZGS 99 was reached 117 days after sowing and after 123 days in the ambient as well as in the elevated [CO_2] treatment.

Effects of the heatwave

The future scenario with both elevated temperature and [CO_2], combined with a 10 day-heatwave of 33/28 °C (day/night) around the reproductive stage, caused a 52 % decrease in grain yield compared to the ambient scenario without heatwave exposure (Table 3). The results also showed that the 10 day-heatwave caused a similar decrease in grain yield, when it was applied in the ambient scenario, as when it was induced in the future two-factor scenario (Fig. 3a).

The applied heatwave decreased grain yield significantly (p>0.001) in all four basic treatments and the strongest decrease was identified under elevated [CO_2] (45 %; Fig. 3a). However, despite this the grain yield after heatwave exposure was still highest at elevated [CO_2] (Table 3). The measured vegetative aboveground biomasses were in all treatments higher after exposure to the heatwave than in the respective basic treatment, although this was only significant under the conditions of ambient and elevated [CO_2] (Table 3). With regard to total aboveground biomass no significant differences were found between any of the treatments (Table 3). Hence, the response patterns for grain yield and vegetative aboveground biomass were almost mirror images of each other in all treatments (Fig. 3a,c,d). Number of ears produced was increased for all treatments after exposure to the heatwave, and consequently reducing the calculated grain yield per ear (Table 3, Fig. 3b). Harvest index was decreased by 58.1 % by the heatwave under elevated [CO_2] and by

39.3 % under elevated temperature (Table 3). The lowest overall HI was found for the future climate scenario of elevated temperature and [CO_2] with an induced heatwave.

Accession specific effects

The first accession to reach ZGS 49 and then transferred to the heatwave was 'Mari' grown under elevated temperature and the last was 'Griechische', - also cultivated under elevated temperature and the time between spanned 35 days. The days to reach ZGS 49 for all accessions in the different basic treatments are shown in Fig. 2. Accessions demonstrating stable rate of development over the four basic treatments were 'Sebastian', 'Solenbyg' and 'Grenoble I' spanning only 2-5 days in reaching ZGS 49. 'Arve', 'Bjørne', 'Brage', 'Brio', 'Mari' and 'Kushteki' were found to be early accessions reaching ZGS 49 as the first ones under all the basic treatments (Fig. 2).

Vast variation in measured production parameters was identified for the 22 accessions when grown under different treatments. The accessions 'Evergreen', 'Brio' and 'Anakin' were less affected by the heatwave than the other accessions in the two-factor treatment +heatwave scenario. They showed the lowest reduction in grain yield and the vegetative aboveground biomass were only moderately affected compared to their production under ambient conditions (Fig. 4). With the exceptions ofs 'Alf', the accessions that were less affected by the two-factor +heatwave treatment, all showed substantial response in grain yield to elevated [CO_2] and showed medium to low grain yield under ambient conditions (Fig. 4, where the accessions are ranked left to right according to their % increased grain yield under elevated [CO_2] compared to ambient). The largest reduction in grain yield was observed in 'Königsberg' and 'Vilm' under the two-factor +heatwave treatment, and with 'Königsberg' demonstrating strongest decrease in vegetative aboveground biomass (Fig. 4).

The accession 'Alliot' showed high resilience towards heatwave exposure both in the ambient treatment and in the future scenario of combined elevated temperature and [CO_2], whereas 'Prestige' also showed resilience to heatwave exposure under ambient conditions, but not under the future combined scenario (Fig. 5). The negative effect of the heatwave within the [CO_2] treatment was strongest for 'Grenoble I' and 'Anita' on grain yield production (Fig. 5). 'Königsberg' responded atypically with regard to grain yield under elevated [CO_2], where no decrease in grain yield due to the heatwave was observed. 'Königsberg' also responded atypically in regard to vegetative aboveground biomass, where it was the only accession that showed reduced vegetative aboveground biomass when exposed to heatwave. The accession 'Brage', which was highest yielding under ambient conditions, was only little affected by the heatwave under elevated temperature, but experienced medium to high decrease in grain yield under the remaining basic treatments with an induced heatwave.

The different responses of the 22 accessions to the climate treatments with and without heatwave resulted in S^2 for grain yield ranging from 1.80 to 9.35, with a low value indicating stability (Table 1). ‘Oslo’ and ‘Prestige’ were found to be the most stable cultivars according to S^2 and ‘Prestige’ also had the seventh highest mean grain yield across treatments, whereas ‘Oslo’ ranked 20th according to mean yield. ‘Brage’ and ‘Kushteki’ were among the top ten cultivars both regarding S^2 and mean grain yield across treatments. In regard to Wricke’s ecovalence for grain yield, W^2 ranged from 2.37 to 23.32 over the eight treatments, where a low score indicates a response pattern corresponding to the majority of the accessions. For W^2 ‘Prestige’ ranked 14th out of the 22 accessions, and it had high grain yield under all climate treatments with and without heatwave, however, with little response to elevated [CO_2]. The accessions ‘Kushteki’, ‘Arve’ and ‘Moscou’ ranked as first, second and third best according to W^2 and ‘Kushteki’ and ‘Moscou’ were found within top ten for mean grain yield across treatments. ‘Grenoble I’, ‘Evergreen’ and ‘Königsberg’ were the three accessions that deviated most in their stability from the rest of the set of accessions. When S^2 and W^2 was calculated over the four basic treatments and compared to S^2 and W^2 of the four heatwave treatments (data not shown), accessions generally demonstrated either stable under the basic treatments or stable under heatwave treatments. ‘Oslo’ was the only exception, as it showed stable grain yield according to S^2 under both the basic treatments and the heatwave treatments.

Discussion

The general trend of having increased grain yield under elevated [CO_2] and decreased under elevated temperature (Lawlor and Mitchell, 1991; Lü *et al*,. 2013) was true also for this study. High variation in degree of decline or increase was though found depending on experimental conditions. However, earlier studies have often concentrated on single-factor treatments and/or limited number of genotypes. For example, FACE conditions at 550 ppm [CO_2] were found to increase grain yield by 9-18 % for barley accession ‘Theresa’ (Manderscheid *et al.*, 2009). A 2 °C temperature increase of the soil (4 cm depth) associated with 4 % yield decline in barley accession ‘Quench’ (Högy *et al.*, 2013). Furthermore, in a phytotron study by Clausen *et al.* (2011) four accessions, (‘Gl. Dansk’, ‘Lazuli’, ‘Anakin’ and ‘Barke’) exposed to [CO_2] at 700 ppm increased grain yield by 57 %, whilst decreased it by 27 % under +5°C compared to the mean of 31 % increase and 40 % decrease presented for 22 accessions in this study, respectively. With regard to the two-factor treatment of constantly elevated temperature and [CO_2], less experimental results have reported the effects on grain yield. In wheat, the combined effect has been reported to affect grain yield negatively (Ziska *et al.*, 1997) and this was also found by modelling (Long *et al.*, 2006). In enclosure studies the grain yield, under the two-factor treatment, could not be predicted from the results of the single-factor treatments (Clausen *et al.*, 2011; Ingvordsen *et al.*, 2014*b*), which was consistent with the present study on barley and previously reported studies on wheat and oilseed rape (Long *et al.*, 2006; Frenck *et al.*, 2011).

The results of the present study revealed a greater penalty on grain yield from the heatwave induced under elevated [CO_2] (45 %) than under elevated temperature (35 %; Fig. 3). Despite the greater decrease in grain yield by the extreme heat under elevated [CO_2], the total production was still increased compared to heatwave exposure under ambient conditions, as was also found in wheat by Bencze *et al.* (2004). The heatwave interfered less with barley plants grown under preceding elevated temperature than in the other environments. This was possibly partly due to acclimation effect induced by the basic treatment with elevated temperature and/or the difference in responses was attributable to the fact that the shift in temperature *per se* was higher and thereby, the temperature shock effect stronger in ambient temperature treatments, when compared to under elevated temperature conditions. A short exposure of plants to high temperature has been shown to limit the effect of a following longer exposure to higher temperature, termed ‘acquired thermotolerance’ (Hamilton *et al.*, 2008).

Production of vegetative aboveground biomass increased under the heatwave scenarios; however, when the total aboveground biomass was considered, the heatwave effect within the climate treatments diminished due to high yield reductions (Fig. 3). Hence, the heatwave changed allocation of the overall production from grain yield to biomass. Such changed allocation patterns were also identified by Batts *et al.* (1998) in one out of two studied wheat cultivars that were exposed to a temperature gradient. The change in allocation found in this study was most prominent under elevated [CO_2], where the heatwave decreased HI by 58 % compared to a reduction of 39-49 % in the other treatments. Even though numerous plant processes are responsible for the allocation of dry matter to the different plant organs, HI has been shown in general to have a high heritability (Hay, 1995) and therefore, grain production under future climate conditions could benefit from identification of genotypes with stable HI across environments or even higher HI between environments.

In the present study the temperature during the extreme heatwave was 9 °C above the temperature in the elevated temperature treatments, but 14 °C above the temperature of the ambient and elevated [CO_2] treatment. Therefore, the more pronounced effect of the heatwave observed under elevated [CO_2] could be an effect of the additional 5°C of temperature increase. However, the greater effect of the heatwave under elevated [CO_2] found in the present study might also result from lower capacity to benefit from elevated [CO_2] in photosynthetic processes as indicated under acute heat stress at the vegetative stage of a C3 crop like barley (Wang *et al.*, 2008; Hamilton *et al.*, 2008). A meta-analysis, contrary reported enhanced net photosynthesis under acute heat stress also at the vegetative stage (Wang *et al.*, 2012). The inconsistencies on the influence to the photosynthesis from severe heat are likely attributable to differences in time of exposure to elevated [CO_2], temperature levels, accession analysed, amounts of water applied and timing of exposure and measurement within plant development. More studies with future elevated levels of both [CO_2] and temperature combined with heatwaves at different time-points during the growth period are needed to understand the effects and consequences of climate change on crop yield.

In the present study the heatwave was applied when first awns were visible and continued for 10 days. Dependent on flowering time of the 22 different spring accessions, the possible most vulnerable time for grain establishment, pollen development and anthesis have been targeted (Sakata *et al.*, 2000; Hakala *et al.*, 2012; Gourdji *et al.*, 2013). Under field conditions the differences in development of the accessions (Fig. 2) together with time of sowing, would have influenced the targeted growth stage of the extreme heatwave. This again highlights the importance of having sufficient variability in cultivar earliness/lateness, and to avoid cultivation of a single dominating cultivar across large areas to enable partial escape from the generally deleteriously harmful effects of heatwaves. Accessions with early flowering could partly escaped the heatwave and thereby potentially maintained grain yield (Tewolde *et al.*, 2006). However, extreme events of the future climate will be unpredictable in frequency and timing, and therefore stability by climate resilient genotypes but also cropping systems (with diverse crop cultivars) must be targeted to avoid large scale crop losses and failures.

Genetic resources to improve resilience to temperature extremes

Resilience should be a central breeding target considering predicted extreme climate events. The 22 spring barley accessions that were analysed here revealed vast variation in response to the eight applied climate treatments, emphasising the need for continually search for the most resilient accessions. The recorded complexity in the response patterns is not, however, apt to simplify the task set for plant breeders. For example, the accessions with better capacity to tolerate heatwave conditions in the ambient treatment were not the same that displayed heatwave-resistance in the scenarios of elevated temperature and [CO_2] (Fig. 5).

Our findings suggest high CO_2-responsiveness, i.e., increased grain yield under elevated [CO_2], to correlate with high yield in the two-factor +heatwave scenario, but not under ambient conditions (Fig. 4). A number of previous studies (Manderscheid and Weigel, 1997; Ziska *et al.*, 2004; Franzaring *et al.*, 2013; Ingvordsen *et al.*, 2014*b*) found no correlation between CO_2-responsiveness and time of cultivar release, which suggests that CO_2-responsivenes has not yet been targeted in breeding.

Surprisingly, the accessions that were high yielding in the two-factor +heatwave treatment often ranked low according to both of the climate-stability scores (high score). Temperature was elevated in six of the eight treatments that were included in the stability measures, which may mean that the stability measurements reflected stability to elevated temperature in particular. When the present findings suggested high CO_2-responsiveness in accessions to correlate with high grain yield in the two-factor +heatwave treatment, the low stability of these accessions can be for the reason that elevated [CO_2] is not as dominating in the stability measurements as elevated temperature.

Numerous environmental and genetic factors influence crop performance during its life cycle and determine the grain yield and vegetative aboveground biomass at long run. Most studies on future abiotic stresses in plants report physiological measures from few genotypes at vegetative stages without relating results to the final yield parameters (Wang *et al.*, 2008; Lü *et al.*, 2013). Increased knowledge on the concerted influence of multiple factors, or how effects reported on vegetative stages are reflected in the final yield is needed for tailoring the future cultivars able to cope with future climatic constraints. As found in this study and also emphasised in few earlier ones (Challinor *et al.*, 2014; Martín *et al.*, 2014) the choice of accession is determining for final production, and this should be reflected in experimental studies.

Uncertainties and limitations

Cultivation of plants in enclosures bias always at some extent the results compared to field conditions. Compromises are, however, necessity as applying extreme weather events to field experiments challenge available experimentation facilities (Kimball *et al.*, 2007; Bruhn *et al.*, 2013). Nevertheless, a selection of the accessions tested in the present study has also been introduced to FACE experiments in Denmark (pers. comm Røjbæk and Jørgensen, DTU-Risø) and the cultivar ranking high by CO_2-responsiveness was in agreement with the findings of this study: e.g., cultivars 'Edvin' and 'Brio' were always among those with the greatest gain in yield under elevated [CO_2].

Under future climate conditions southern Scandinavia can experience decreased summer precipitation (IPCC, 2007). However in the present study drought is only indirectly included since water was applied in all treatments according to the amount appropriate for optimal development under ambient conditions. In the treatments of elevated temperature and under heatwave conditions increased vapor pressure deficit has despite equal relative humidity between treatments, changed evapotranspiration conditions from treatments of ambient temperature. Therefore, reported effects of the treatments with elevated temperature can be concerted by heat and drought responses. A 10 day-heatwave could occur with no precipitation. However, in the field, crops could take up water from an area of soil not determined by a pot, and dependent on soil type, there could be more water available to limit the effect on grain yield compared to enclosure studies.

Conclusion

The identified variation in the set of barley accessions in response to multifactor climate treatments was high as such, however, not sufficient, when considering future ability to cope with heatwaves and avoid severe yield losses or total failures. In fact, these results emphasized the threat that temperature extremes exerts on crop production systems. Responsiveness of barley accessions to elevated [CO_2] tended to correlate with high grain yield in the future scenario of elevated temperature and [CO_2] + 10 day-heatwave at around flowering. Experimental studies assessing grain yield under multifactor climate conditions with superimposed variability should be encouraged to increase basic understanding and to identify genes and genotypes for future breeding programs, when aiming to breed for more resilient and high yielding set of cultivars.

Supplementary data

The supplementary data is available in print in the dissertation and digitally on the attached CD-rom.

Supplementary Table 1. Additional information on the 22 accessions included in the study

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Table 1. The accessions included in the present study and found their stability over applied treatments of future levels of [CO_2], temperature, their combination and heatwave. Cultivar (CV), environmental variance (S^2), genebank number (NGB), landrace (LR) and Wricke's ecovalence (W^2). The accessions are sorted after the mean grain yield across the eight treatments (m_i) and numbers in brackets are the ranking from their stability indices.

Accessions name	NGB / Breeder	Culton type	Sub type	Country of origin / Country of breeding	Year of release	m_i	S^2	W^2
Bjørne	NGB9326	LR	6	unknown		6.25	6.19(17)	5.23(6)
Evergreen	Nordic Seed A/S Plant Breeding	CV	2	Denmark	2010	6.19	9.35(21)	17.46(20)
Brio	NGB9327	CV	6	Sweden	1924	6.13	6.04(15)	5.93(9)
Brage	Graminor Plant Breeding	CV	6	Norway	2010	5.97	5.15(7)	5.89(8)
Anakin	Sejet Plant Breeding I/S	CV	2	Denmark	2006	5.91	5.83(11)	5.09(5)
Solenbyg	NGB13402	LR	6	Norway		5.90	7.38(18)	8.41(13)
Prestige	NGB16750	CV	2	France	2000	5.88	2.68(2)	9.00(14)
Kushteki	NGB6288	LR	6	Afghanistan		5.87	5.48(9)	2.37(1)
Moscou	NGB9482	LR	2	unknown		5.83	5.93(13)	4.00(3)
Drost P.	NGB6281	CV	2	Denmark	1951	nn	nn	nn
Alliot	NGB16757	CV	2	Denmark	1999	5.73	4.42(6)	15.03(18)
Sebastian	Sejet Plant Breeding I/S	CV	2	Denmark	2002	5.72	3.89(4)	5.98(10)
Griechische	NGB9333	LR	6	Greece		5.56	5.57(10)	4.11(4)
Arve	NGB11311	CV	6	Norway	1990	5.36	5.90(12)	3.72(2)
Grenoble I	NGB9378	LR	6	France		4.63	8.67(20)	23.32(21)
Edvin	Boreal Plant Breeding	CV	6	Finland	2008	4.54	7.95(19)	11.76(16)
Vilm	NGB9435	LR	2	Germany		4.21	5.98(14)	5.34(7)
Anita	NGB15250	CV	6	Norway	1962	4.20	6.15(16)	7.27(12)
Mari	NGB1491	CV	2	Sweden	1960	4.12	5.36(8)	12.03(17)
Oslo	NGB9315	LR	6	Norway		4.09	1.80(1)	10.25(15)
Königsberg	NGB9310	LR	6	unknown		3.65	3.12(3)	16.71(19)
Alf	NGB4707	CV	2	Denmark	1978	3.41	4.20(5)	6.32(11)

Table 2. Experimental levels of temperature (tmp), [CO_2] (CO_2) and humidity in applied treatments of four basic treatments and two heatwave treatments (+H) treatments ± standard deviations.

	Temp day/night		CO_2 (constant)		**humidity day/night**	
	target	experimental	target	experimental	target	experimental
ambient	19/12°C	18.36±2.18/13.02±2.48	400 ppm	451.54±40.65	55/70	56.55±4.53/67.43±5.41
+CO_2	19/12°C	18.22±2.23/13.04±2.45	700 ppm	700.27±23.18	55/70	56.63±4.65/67.51±5.38
+tmp	24/17°C	22.97±2.18/18.20±2.45	400 ppm	459.72±40.85	55/70	56.64±4.61/67.25±5.43
+tmp & CO_2	24/17°C	23.05±2.24/18.08±2.44	700 ppm	693.89±23.76	55/70	56.66±4.69/67.32±5.42
+H	33/28°C	32.41±2.63/28.01±2.52	400 ppm	446.88±19.09	55/70	56.47±4.41/67.59±5.24
+H & +CO_2	33/28°C	32.42±2.52/28.02±2.50	700 ppm	703.14±21.00	55/70	56.43±4.55/67.55±5.20

Table 3. Overall production parameters averaged from 22 spring barley accessions cultivated under ambient (amb) or elevated levels of [CO_2] (+CO_2) and temperature (+tmp) with (+H) and without a 10 day-heatwave. In columns of +H, '*' indicates significance between the heatwave treatment (+H) and the corresponding treatment without heatwave (ctrl) and in columns of ctrl, '*' indicates significance between the treatment and this production parameter under ambient conditions.

	amb		**+tmp & CO_2**		**+tmp**		**+CO_2**	
	ctrl	+H	ctrl	+H	ctrl	+H	ctrl	+H
Grain yield (g plant^{-1})	7.01±1.13	4.45±1.48***	5.75±1.37**	3.35±1.18***	4.22±1.06***	2.71±0.99***	9.16±1.75***	5.01±1.78***
Total ears (no plant^{-1})	6.37±2.65	11.08±5.13***	8.24±2.78*	13.77±5.33***	7.01±3.10	11.40±6.18**	6.30±1.75	13.42±6.22***
Grain yield per ear (g ear^{-1})	1.28±0.54	0.50±0.29***	0.80±0.34**	0.29±0.17***	0.59±0.21***	0.27±0.12***	1.59±0.58	0.46±0.29***
Vegetative aboveground biomass (g plant^{-1})	8.48±1.75	10.15±1.68**	10.12±1.97**	11.24±2.58	7.81±1.88	8.24±2.09	11.08±1.93***	14.46±2.47***
Total aboveground biomass (g plant^{-1})	15.49±2.08	14.60±2.68	15.87±2.64	14.59±2.62	12.03±2.37***	10.95±2.60	20.24±2.22***	19.46±3.01
HI (%)	86.30±0.23	43.90±0.14***	58.50±0.16***	31.80±0.14***	56.30±0.16***	33.80±0.13***	85.70±0.22	35.80±0.14***

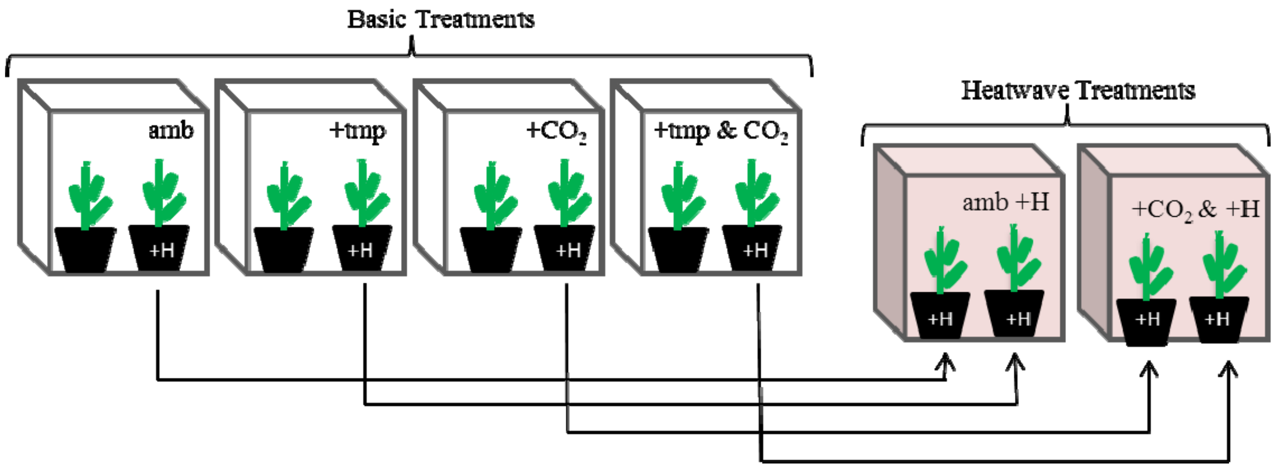

Fig. 1. Schematic overview of basic treatments and extreme heatwave treatments. Amb: ambient conditions, $+CO_2$: elevated [CO_2] conditions, +tmp: elevated temperature conditions, +tmp & CO_2: elevated temperature and [CO_2] conditions, +H: heatwave applied.

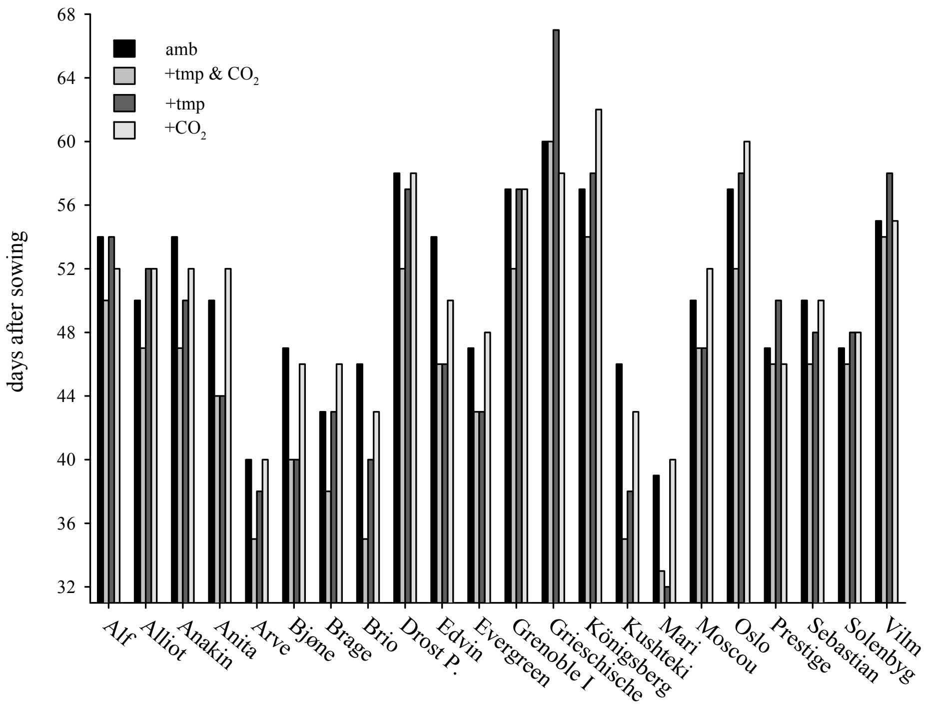

Fig. 2. Days after sowing of the accessions in the four basic treatment before transfer to heatwave treatment . An accession was transferred when four of eight plants reached Zadoks growth stage 49. Ambient conditions: amb, elevated [CO_2]: +CO_2, elevated temperature: +tmp and elevated [CO_2] and temperature in combination: +tmp & CO_2.

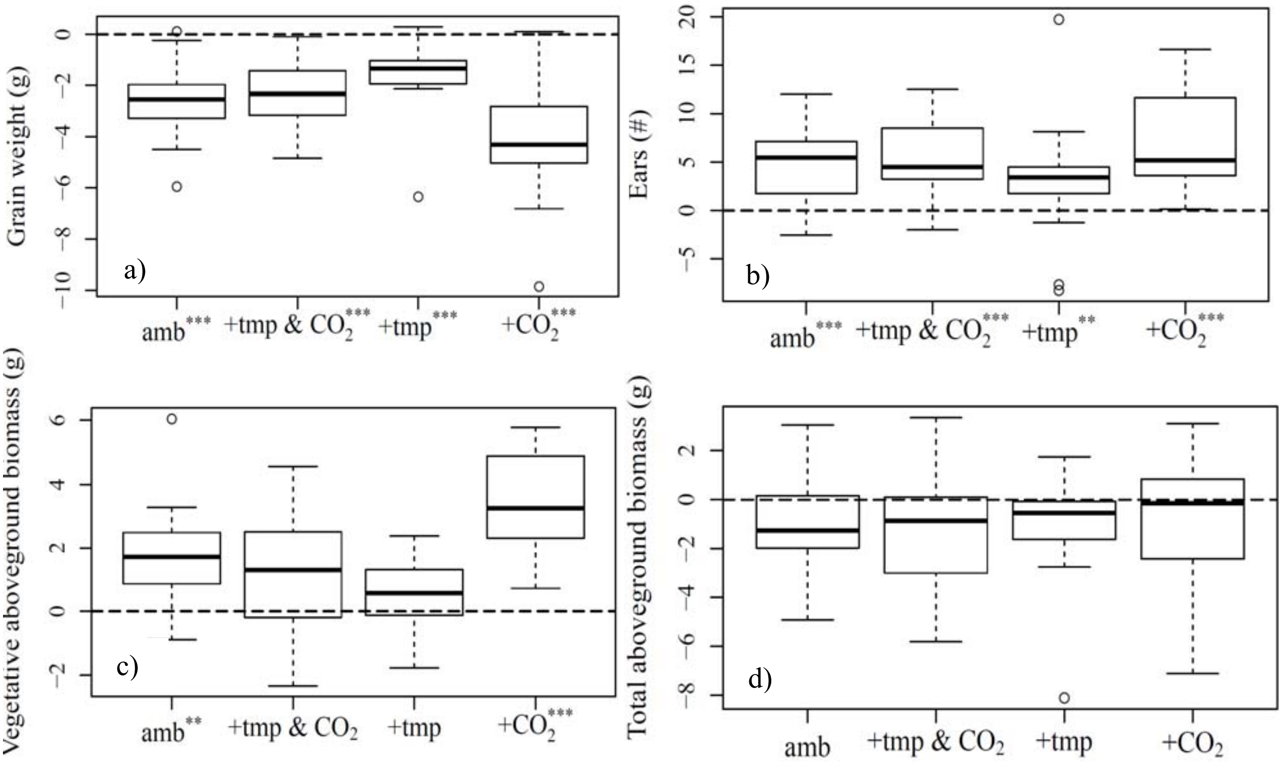

Fig. 3. Effect of the heatwave within the basic treatments of ambient (amb), elevated temperature and [CO_2] (+tmp & CO_2), elevated temperature (+tmp) and elevated [CO_2] (+CO_2) on grain yield (a), ears (b), vegetative aboveground biomass (c) and total aboveground biomass (d). Dotted line (----) is the production in the corresponding control treatment in a no-heatwave scenario.

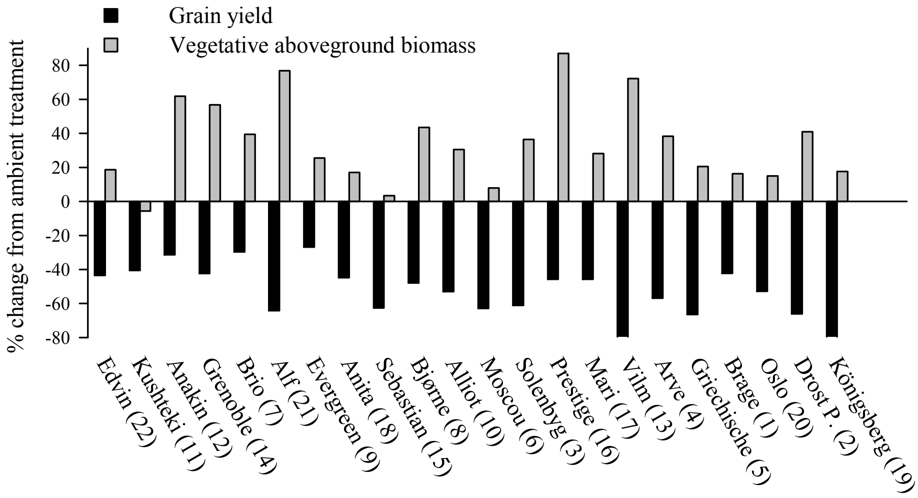

Fig. 4. Change (%) in grain yield and vegetative aboveground biomass of 22 spring barley accessions exposed to a 10 day-heatwave around flowering in the scenario of combined elevated temperature and [CO_2]. Results are given relative to ambient conditions. The accessions are ordered according to their grain yield under elevated [CO_2] (% relative to ambient), with decreasing yield from left to right. In brackets is the rank for grain yield under basic ambient conditions.

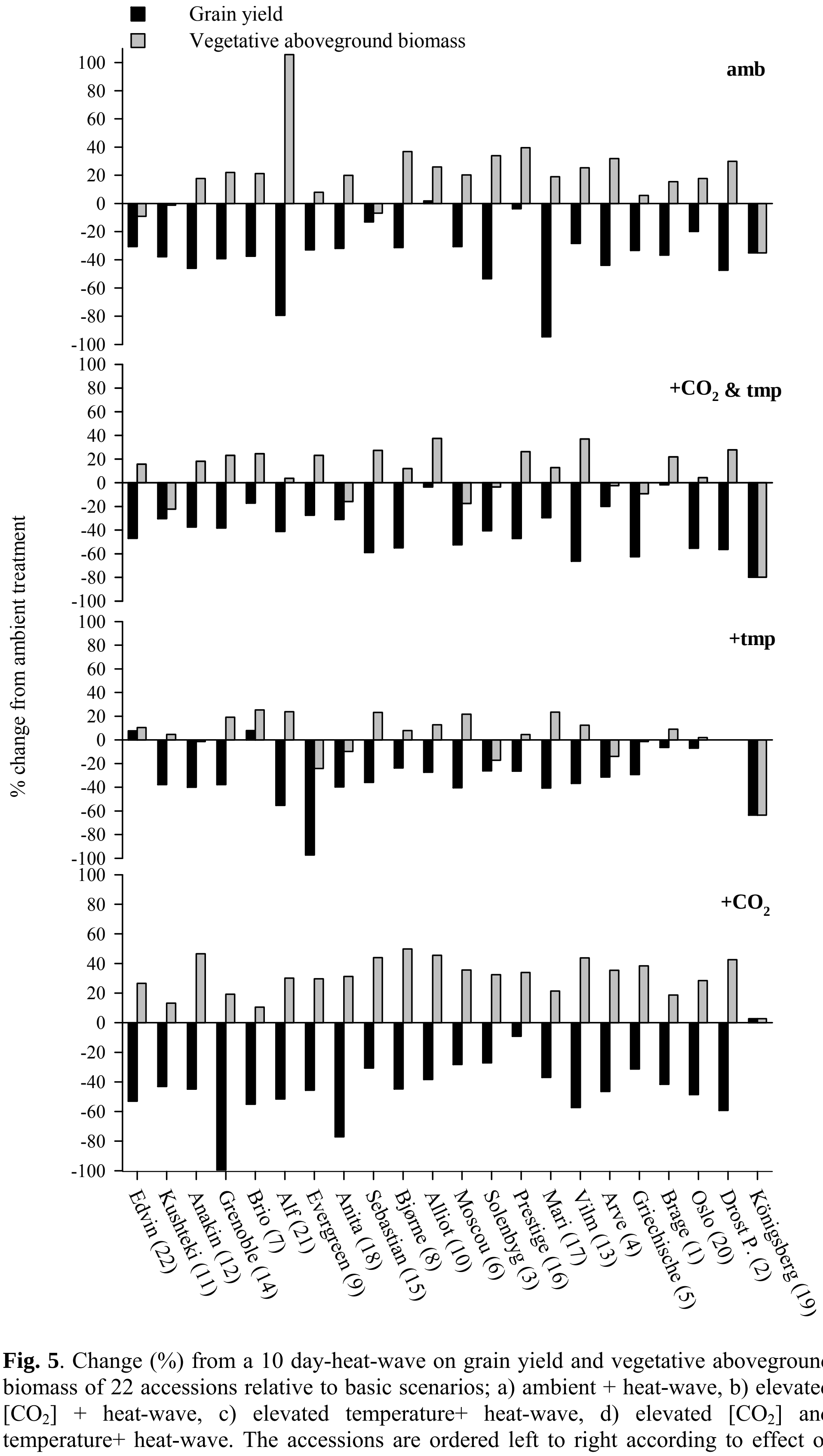

Fig. 5. Change (%) from a 10 day-heat-wave on grain yield and vegetative aboveground biomass of 22 accessions relative to basic scenarios; a) ambient + heat-wave, b) elevated [CO_2] + heat-wave, c) elevated temperature+ heat-wave, d) elevated [CO_2] and temperature+ heat-wave. The accessions are ordered left to right according to effect of heat-wave on grain yield in the ambient treatment. In brackets is the rank for grain yield under basic ambient conditions.

Supplementary material

Manuscript 4

Supplementary data

Table S1. Additional information on accessions in the study.

Accession name used	Accession name	NordGen no.	Cultivar type	Origin country/ Breeding country	Sub type (row)	Collecting date / Release year	Supplied by
Alf	Alf	NGB4707	CV	Denmark	2	1978	NordGen
Alliot	Alliot	NGB16757	CV	Denmark	2	1999	Nordic Seed A/S Plant Breeding
Anakin	Anakin		CV	Denmark	2	2006	Sejet Plant Breeding I/S
Anita	Anita	NGB15250	CV	Norway	6	1962	NordGen
Arve	Arve	NGB11311	CV	Norway	6	1990	NordGen
Brage	Brage		CV	Norway	6	2010	Graminor Plant Breeding
Brio	Brio	NGB9327	CV	Sweden	6	1924	NordGen
Drost P	Drost Pajbjerg	NGB6281	CV	Denmark	2	1951	NordGen
Edvin	Edvin		CV	Finland	6	2008	Boreal Plant Breeding
Evergreen	Evergreen		CV	Denmark	2	2010	Nordic Seed A/S Plant Breeding
Mari	Mari	NGB1491	CV	Sweden	2	1960	NordGen
Prestige	Prestige	NGB16750	CV	France	2	2000	The Danish AgriFish Agency, Tystofte
Sebastian	Sebastian		CV	Denmark	2	2002	NordGen
Bjørne	Bjørne	NGB9326	L		6		NordGen
Grenoble I	Grenoble I KVL 131	NGB9378	L	France	6		NordGen
Griechische	Griechische KVL 56	NGB9333	L	Greece	6		NordGen
Königsberg	Königsberg KVL 18	NGB9310	L		6		NordGen
Kushteki	Kushteki K.77	NGB6288	L	Afghanistan	6	1948----	NordGen
Moscou	Moscou KVL 353	NGB9482	L		2		NordGen
Oslo	Oslo KVL 25	NGB9315	L	Norway	6		NordGen
Solenbyg	Solenbyg	NGB13402	L	Norway	6		NordGen
Vilm	Vilm KVL 248	NGB9435	L	Germany	2		NordGen

Accession name	Location / Breeder institute	Culton pedigree
Alf	Nordic Seed A/S Plant Breeding	Mutant in Bomi
Alliot	Nordic Seed A/S Plant Breeding	Waggon*Imidis
Anakin	Sejet Plant Breeding I/S	Tumbler x Respons
Anita	Norwegian Agrcultural University	HOL-44 x Arve; / (Asplund x Ds295) x Varde
Arve	Graminor Plant Breeding	(Otra x Vigdis) x Agneta
Brage	Graminor Plant Breeding	Lavrans/NK91650

Accession name	Location / Breeder institute	Culton pedigree
Brio	Sveriges Utsadesforening Svalöf	Selection from 6-row barley from Skåne
Drost Pajbjerg	The Pajbjerg Foundation	Maja x Kenia
Edvin	Boreal Plant Breeding	Verner/Hja 85194
Evergreen	Nordic Seed A/S Plant Breeding	Br6920b115*Quench
Mari	Sveriges Utsadesforening Svalöf	Mutation selected from X-ray treated Bonus
Prestige	RAGT	AC 00/767/5 x Anakin
Sebastian	Sejet Plant Breeding I/S	Lux x Viskosa
Bjørne		
Grenoble I KVL 131		
Griechische KVL 56		
Königsberg KVL 18		
Kushteki K.77	Kushteki	
Moscou KVL 353		
Oslo KVL 25		
Solenbyg		
Vilm KVL 248		

Joint author statements

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Joint author statement

If a thesis contains articles (i.e. published journal and conference articles, unpublished manuscripts, chapters etc.) made in collaboration with other researchers, a joint author statement about the PhD student's part of each article shall be made by a representative sample of authors comprised of: 1) *Corresponding author and/or principal/first author (defined by PhD student)*, and 2) *1-2 non-DTU authors (non-supervisor authors)*. *If relevant – alternatively all*, cf. article 12, section 4 and 5 of the Ministerial Order No. 1039 27 August 2013 about the PhD degree. We refer to the Vancouver protocol's definition of authorship.

Titel of the article	Significant decrease in yield under future climate conditions: Stability and production of 138 spring barley accessions
Author(s)	Ingvordsen C H, Backes G, Lyngkjær M F, Peltonen-Sainio P, Jensen J D, Jalli M, Jahoor A, Rasmussen M, Mikkelsen T N & Jørgensen R B
Journal/conference * If applicable	Submitted to European Journal of Agronomy March 2014
Name of PhD student	Cathrine Heinz Ingvordsen
Date of Birth	0807-1982

Description of the PhD student's contribution to the abovementioned article

Cathrine main contributed to experimental work, data analysis and was lead author

Signature of the PhD student [signature] Date 25/4 - 14

Signatures of co-authors

As a co author I state that the description given above is the best of my knowledge corresponds to the process and I have no further comments

Date (DD/MM/YY)	Name	Signature
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Joint author statements shall be delivered to the PhD administration along with the PhD thesis

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Joint author statement

If a thesis contains articles (i.e. published journal and conference articles, unpublished manuscripts, chapters etc.) made in collaboration with other researchers, a joint author statement about the PhD student's part of each article shall be made by a representative sample of authors comprised of: 1) *Corresponding author and/or principal/first author (defined by PhD student)*, and 2) *1-2 non-DTU authors (non-supervisor authors)*, *if relevant – alternatively all* cf. article 12, section 4 and 5 of the Ministerial Order No. 1039 27 August 2013 about the PhD degree. We refer to the Vancouver protocol's definition of authorship.

Titel of the article	**GWAS of production and stability traits in spring barley cultivated under future climate scenarios**
Author(s)	**Ingvordsen C H, Backes G, Lyngkjær M F, Peltonen-Sainio P, Jahoor A, Mikkelsen T N & Jørgensen R B**
Journal/conference (if applicable)	**Aimed at Molecular Breeding**
Name of PhD student	**Cathrine Heinz Ingvordsen**
Date of Birth	**0607-1982**

Description of the PhD student's contribution to the abovementioned article

Cathrine main contributed to experimental work, data analysis and was lead author

Signature of the PhD student [signature] **Date** 25/4-14

Signatures of co-authors

As a co author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

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25/4/14	**Rikke B. Jørgensen**	[signature]

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If a thesis contains articles (i.e. published journal and conference articles, unpublished manuscripts, chapters etc.) made in collaboration with other researchers, a joint author statement about the PhD student's part of each article shall be made by a representative sample of authors comprised of: 1) *Corresponding author and/or principal/first author (defined by PhD student)*, and 2) *1-2 non-DTU authors (non-supervisor authors), if relevant – alternatively all*, cf. article 12, section 4 and 5 of the Ministerial Order No. 1039 27 August 2013 about the PhD degree. We refer to the Vancouver protocol's definition of authorship.

Titel of the article	Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 barley (Hordeum vulgare L.) accessions
Author(s)	Ingvordsen C H,Gislum R, Jørgensen J R, Mikkelsen T N & Jørgensen R B
Journal/conference * if applicable	Aimed at Journal of Agricultural and Food Chemistry
Name of PhD student	Cathrine Heinz Ingvordsen
Date of Birth	0607-1982

Description of the PhD student's contribution to the abovementioned article

Cathrine H Ingvordsen performed the experimental work, data analysis and was lead author. The only part of the present work not performed by Cathrine H Ingvordsen was statistical handling of the NIR spectra and the extrapolation for prediction of protein concentration.

Signature of the PhD student Date 25/4-14

Signatures of co-authors

As a co-author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

Date (DD/MM/YY)	Name	Signature
23/04/2014	René Gislum	
23/04/2014	Johannes R. Jørgensen	
25/4/14	Teis N. Mikkelsen	
23/04/2014	Rikke B. Jørgensen	

Joint author statements shall be delivered to the *PhD administration* along with the PhD thesis

Joint author statement

If a thesis contains articles (i.e. published journal and conference articles unpublished manuscripts, chapters etc) made in collaboration with other researchers, a joint author statement about the PhD student's part of each article shall be made by a representative sample of authors comprised of: 1) *Corresponding author and/or principal/first author (defined by PhD student)*, and 2) *1-2 non-DTU authors (non-supervisor authors). if relevant – alternatively all*, cf article 12, section 4 and 5 of the Ministerial Order No. 1039 27 August 2013 about the PhD degree. We refer to the Vancouver protocol's definition of authorship.

Titel of the article	**Effect of an extreme heatwave on 22 spring barley accessions cultivated in future climates. Tendencies in allocation of biomass, temperature priming, CO2-responsiveness and stability of grain yield**
Author(s)	**Ingvordsen C H, Lyngkjær M F, Peltonen-Sainio P, Mikkelsen T N & Jørgensen R B**
Journal/conference * if applicable	**Aimed at Journal of Experimental Botany/Global Climate Change**
Name of PhD student	**Cathrine Heinz Ingvordsen**
Date of Birth	**0607-1982**

Description of the PhD student's contribution to the abovementioned article

Cathrine main contributed to experimental work, data analysis and was lead author

Signature of the PhD student Cathrine Heinz **Date** 25/4-'14

Signatures of co-authors

As a co-author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

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29/4/14	**Michael F. Lyngkjær**	
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25/4/14	**Teis N. Mikkelsen**	
25/4/14	**Rikke B. Jørgensen**	

Joint author statements shall be delivered to the *PhD administration* along with the PhD thesis

MANUSCRIPTS IN PREP.

- excluded from evaluation

- Ingvordsen CH, Backes G, Jalli M, Reitan L, Jensen JD, Lyngkjær MF, Peltonen-Sainio P, Jørgensen RB **Marker-trait associations in spring barley for leaf rust, net blotch, ramularia, scald and spot blotch detected by genome-wide association**

- Helle Bøg, Per Ambus, Ingvordsen CH, Mikkelsen TN, Lyngkjær MF, Backes G, Jørgensen RB **Field comparison of spring barley cultivars exposed to increased [CO_2]**

- A.M. Torp, C.H. Ingvordsen, R.B. Jørgensen & S.K. Rasmussen **Effects of the changing climate on the quality of barley seeds**

- Niero M, Ingvordsen CH, Hauschild MZ, Lyngkjær MF, Peltonen-Sainio P, Jørgensen RB **Eco-efficient production of spring barley in a changed climate: a Life Cycle Assessment including primary data from future climate scenarios**
  The manuscript has been submitted to manuscript has been submitted to Global Change Biology April 2014.

Marker-trait associations in spring barley for leaf rust, net blotch, ramularia, scald and spot blotch detected by genome-wide association

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Abstract

Biotic stresses is expected to decrease global food production by at least one-third under the management conditions of today, where $32 billion dollars annually is spent on pesticides. Further, yield loss caused by plant diseases alone is estimated to be 10 %. In this context one approach is to develop resistant crops. Disease resistant crops have the advantage that they are environmentally sound and save the cost of pesticides. In preset study, 138 predominantly Nordic accessions of spring barley – from landraces to breeder-lines – were cultivated at Nordic breeding stations and scored for infection of either leaf rust, net blotch, ramularia, scald or spot blotch. The phenotypes were included in a genome-wide association study with genotypes revealed by 7864 SNP markers (Illumina, SNP-array). The marker-trait associations were identified using a compressed mixed linear model with the GAPIT package, and conservative validation of markers was performed to avoid false positives. Novel marker-trait associations are reported for ramularia on 1H and spot blotch at the adult stage on 4H. In addition, marker-trait associations were identified for rust on 3H, net blotch at the adult stage on 3H, 5H and 6H and for scald on 7H. Markers for use in resistance breeding are reported, and the co-localising of interesting genes with the SNP-markers is discussed.

Manuscript in prep.

Field comparison of spring barley cultivars exposed to increased [CO_2]

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Abstract

A total of 10 cultivars of spring barley (*Hordeum vulgare* L.) were cultivated season-long in the field at ambient (396 ppm) and above ambient (510 ppm) CO2 concentrations in a Free Air Carbon Enrichment (FACE) facility. The cultivars were 2- as well as 6-rowed types that were marketed in the period 1924-2010. The production parameters grain yield, above ground vegetative biomass, harvest index, thousand grain weight, and plant height were analyzed. Besides, progression of fungal diseases was observed over the growth season. Water use efficiency in a subset of the cultivars was assessed by measurements of the 13C/12C isotope ratio in leaf material. The 10 cultivars had earlier been cultivated at ambient and elevated [CO2] in a highly controlled climate phytotron, and their grain yield and above ground vegetative biomass were compared among the FACE facility and the climate phytotron. The results showed that three cultivars were consistently found among the top-five productive cultivars both in the phytotron and in the FACE-facility. Also, some of the cultivars, which increased their grain yield the most in response to elevated [CO2] were the same in both environments. Of the ten cultivars evaluated under FACE conditions, 'Evergreen' had the highest grain yield at both ambient and above ambient [CO2] levels, and also a high Δ13C value indicating good water use efficiency

The FACE facility applied consisted of eight octagons, four of which were fumigated with extra CO2. CO2 was distributed to the octagons from height-adjustable horizontal pipes in the upwind-direction. A model was developed for a more precise estimation of the [CO2] at different parts of the FACE octagons over the growing season.

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Effects of the changing climate on the quality of barley seeds

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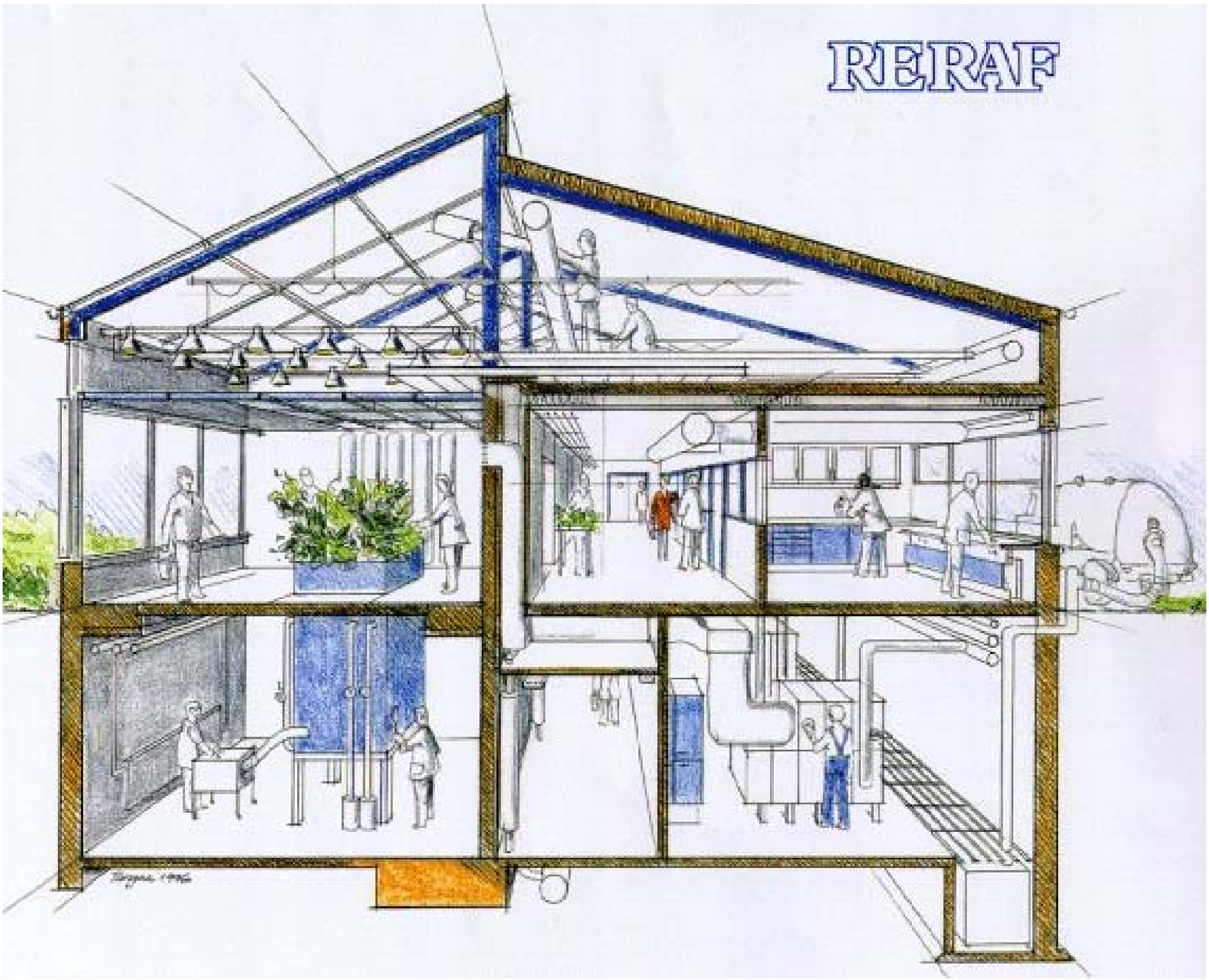
Not only productivity but also quality of crops are likely to be affected by the predicted climate change, however, little is known about the magnitude of effects from the changing climate on crop quality in barley. The mature seed quality of 22 spring-type barley landraces and varieties exposed to elevated temperature (24/19 ^{O}C day/night), [CO_2] (700 ppm) and [O_3] (100-150 ppb) as single factors, as well as a combination of elevated temperature and [CO_2] were evaluated and compared to a control treatment mimicking present Danish early summer conditions (ambient, 19/12 ^{O}C day/night, 387 ppm CO_2, O_3 ot added). Elevated CO_2 concentrations did not significantly change the concentration of phytic acid (PA-P) compared to the ambient treatment, whereas the concentration was significantly higher for the three other treatments elevated [O_3], elevated temperature and the combination of elevated temperature and [CO_2]. The change in PA-P concentration was most likely caused by changes in seed weight as the average content of PA-P per grain was unaffected by treatment. In addition, there were no significant differences in the percentage of total P stored as phytic acid among the treatments. The concentration of iron (Fe) and zink (Zn) in the seed was significantly lower under elevated CO_2 levels compared to all other treatments and decreased by 38% and 29%, respectively, compared to the ambient treatment. This difference could not be entirely explained by changes in seed weight. Bioavailability of Fe determined by the molar ratio between PA-P and Fe was poor and averaged 10.9 under ambient conditions and was significantly impaired under all other treatments. For Zn the ratio averaged 21.7 under ambient conditions and only elevated CO_2 levels showed a significant negative effect (average Phytic acid: Zn ratio = 28.8) on bioavailability of Zn. The main effect of genotypes were generally relatively low and of less importance than interactions between genotypes and treatments. Taken together results from the present study indicates that it may be possible to identify genotypes that are relatively stable across treatments and show above average yield and quality under the combination of elevated temperature and [CO_2].

Manuscript in prep.



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