Alleles of adaptive importance for barley cultivation in a subarctic climate

Alleler av adaptiv betydning for byggdyrking i subarktisk klima

Samsætur mikilvægar fyrir aðlögun byggs að norðurslóðum

Philosophiae Doctor (PhD) Thesis

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List of papers

Paper I

Göransson, M., Hallsson, J.H., Lillemo, M., Orabi, J., Backes, G., Jahoor, A., Hermannsson, J., Christerson, T., Tuvesson, S., Gertsson, B., Reitan, L., Alsheikh, M., Aikasalo, R., Isolahti, M., Veteläinen, M., Jalli, M., Krusell, L., Hjortshøj, R.L., Eriksen, B., Bengtsson, T. (2019) **Identification of ideal allele combinations for the adaptation of spring barley to Northern latitudes.** *Frontiers in Plant Science* 10:542. https://doi.org/10.3389/fpls.2019.00542

Paper II

Göransson, M., Hallsson, J.H., Bengtsson, T., Bjørnstad, Å., Lillemo, M. (2021) **Specific Adaptation for Early Maturity and Height Stability in Icelandic Spring Barley.** *Crop Science* 61:2306-2322. https://doi.org/10.1002/csc2.20459

Paper III

Göransson, M., Sigurðardóttir, Þ.H., Lillemo, M., Bengtsson, T., Hallsson, J.H. (2021) **The winter type allele of *HvCEN* is associated with earliness without severe yield penalty in Icelandic spring barley (*Hordeum vulgare* L.).** *Frontiers in Plant Science* 12: 1801. https://doi.org/10.3389/fpls.2021.720238

Abstract

Barley is the most grown cereal in the Nordic countries, and the fourth most important cereal crop globally. Its use is mainly for feed, but a large portion is also used by the brewing industry. At northern latitudes, such as Iceland, the barley production goes almost exclusively to feed. There is a potential for a diversification of the end-use, but this is dependent on the quality of the produce.

The ability of spring barley to mature in cold temperature is of agronomic importance for cultivation in Iceland. Summer temperature in Iceland is on average lower than at comparable latitudes which often makes it impossible for barley to reach full maturity. This negatively impacts grain quality, and necessitates grain drying before storage, which adds considerable cost for the producer.

In this study, Icelandic early genotypes were grown alongside a panel comprising the current Nordic barley gene pool in multi-location field trials and genome wide association studies (GWAS) were used to elucidate loci of importance for heat sum to heading, heat sum to maturity, and straw length. Nordic barley was studied in controlled environments of contrasting day length and temperature conditions to elucidate the effect on heading, maturity, and straw length. GWAS was used to find marker trait associations. A panel of genotypes were selected for an in-depth study of allelic diversity in the four earliness genes *Ppd-H1*, *HvELF3*, *HvCEN*, and *HvFT1*.

An allele combination from the three most significant marker-trait associations (MTAs) had a 214°dC lower heat sum requirement to maturity, corresponding to 30 days in Iceland. Several loci of adaptive importance in Icelandic conditions were found, including *HvELF3*, *HvGA20ox2*, and *HvGA20ox1*.

The winter allele of *Ppd-H1*, in combination with a *HvFT1* allele, caused extreme earliness in Icelandic spring barley, however with a severe yield penalty. The winter-type allele of *HvCEN* was unique among a set of Icelandic and Finnish genotypes with the ability to combine earliness with high yield. Earlier studies have found a latitudinal gradient in the allelic diversity in both *Ppd-H1* and *HvCEN.* In genotypes adapted to the extreme north, the pattern of allelic diversity at *Ppd-H1* and *HvCEN* was contrasting to what has earlier been reported from European spring barley.

Knowledge of the allelic diversity associated with tolerance to low temperature during the growth season opens the possibility to increase the quality of the grain, which in turn would enable a diversification of the end-use to include products such as malt, and grain used for human consumption. The knowledge of the genetics behind barley with low heat sum requirement to maturity paves the way for better precision and increased speed in the selection process of new breeding lines adapted to the northern margin of barley cultivation. This will enable an expanded production at high latitudes, in Iceland and beyond.

Sammendrag

Bygg er den mest dyrkede kornarten i Norden, og den fjerde viktigste kornarten globalt. Bygg anvendes hovedsakelig til fôr men en stor andel går til malt. Evnen til å modne ved lav temperatur er av agronomisk betydning for vårbyggdyrking på Island. Sommertemperaturen på Island er lavere enn på sammenlignbare breddegrader, noe som gjør det vanskelig for bygg å nå full modning. Dette påvirker kornkvaliteten negativt, og bøndene må tørke kornet før lagring, noe som er en ekstra kostnad for produsentene.

I denne studien har vi dyrket islandske tidlige genotyper ved siden av nåværende nordiske byggsorter og foredlingslinjer i feltforsøk på flere steder og gjennomført assosiasjonskartlegging for å belyse hvilke gener som er av betydning for tidlighet (blomstring og modning) og strålengde. Vi dyrket videre nordiske byggsorter under kontrollerte betingelser med kontraster i daglengde og temperatur for å studere effekten på tidlighet og strålengde. Assosiasjonskartlegging ble brukt for å finne genetiske markører assosiert med disse egenskapene. Vi valgte også et sett med bygglinjer for å studere allelisk diversitet i de fire tidlighetsgenene *Ppd-H1*, *HvELF3*, *HvCEN* og *HvFT1*.

Vi fant en haplotype fra de tre mest signifikante assosiasjonene som hadde et 214°dC lavere varmesumbehov til modning, tilsvarende 30 dager på Island. Vi fant videre et sett med gener som er av adaptiv betydning for islandske forhold, inkludert *HvELF3*, *HvGA20ox2* og *HvGA20ox1*.

Vi fant at vinterallelet til *Ppd-H1*, i kombinasjon med et *HvFT1*-allel, forårsaket ekstrem tidlighet i islandsk vårbygg, dog med et alvorligt avlingstap. Vi fant at vintertype-allelet til *HvCEN* er unikt blant et sett av islandske og finske genotyper med evnen til å kombinere tidlighet med høy avling. Tidligere studier har funnet en breddegradsgradient i det alleliske mangfoldet i både *Ppd-H1* og *HvCEN*. I genotyper adaptert til den nordlige grensen for mulig byggdyrking var mønsteret for allelisk diversitet i *Ppd-H1* og *HvCEN* forskjellig fra hva som tidligere er rapportert fra europeisk vårbygg. Denne kunnskapen legger til rette for bedre presisjon og raskere utvalg av nye foredlingslinjer tilpasset den nordlige grensen for byggdyrking.

Kunnskap om alleldiversiteten som påvirker toleransen for lav temperatur under vekstsesongen åpner muligheter til å forbedre kvaliteten på kornet, hvilket i sin tur vil gi flere muligheter til å bruke avlingen, f.eks til malt og matvarer. Kunnskap om allelene som tilpasser bygg til å modne i lav temperatur gir muligheter for bedre presisjon og hurtigere seleksjon av foredlingsmateriale tilpasset nordlige forhold. Dette vil være til hjelp for å øke produksjonen på nordlige breddegrader, på Island såvel som i andre nordlige strøk.

Úrdráttur

Bygg er mest ræktaða korntegundin á Norðurlöndunum, og er fjórða mikilvægasta korntegundin á heimsvísu. Notkun byggs er mest sem fóður en er einnig mikið notað í bruggun. Á norðurslóðum, þar með talið á Íslandi, er bygg nánast einvörðungu ræktað sem fóður. Sóknarfæri felast í því að auka fjölbreytni í notkun byggs en það er háð gæðum uppskerunnar.

Eiginleiki vorbyggs til að þroskast við lágt hitastig er nauðsynlegur til þess að ræktun sé möguleg á Íslandi. Sumarhiti á Íslandi er töluvert lægri en á svipuðum breiddargráðum og þar af leiðandi nær bygg sjaldnast fullum þroska á Íslandi. Þetta hefur neikvæð áhrif á gæði uppskerunnar og krefst oft á tíðum þurrkunar áður en hægt er að geyma uppskeruna sem eykur kostnað framleiðenda.

Í þessu verkefni voru íslenskar byggarfgerðir ræktaðar samhliða úrvali norrænna byggarfgerða í fjölda tilrauna á Norðurlöndunum og víðar og erfðamengis tengslagreiningar (Genome wide association studies, GWAS) notaðar til að bera kennsl á genasæti tengd skriði, þroskun og hæð byggs með hliðsjón af hitasummu. Norrænar arfgerðir byggs voru rannsakaðar í stýrðu umhverfi þar sem áhrif daglengdar og hitastigs á skriðdag, þroska og hæð voru könnuð. GWAS var notað til að finna tengsl milli erfðamarka og eiginleika. Úrval arfgerða voru valdar fyrir ítarlegri greiningu á samsætufjölbreytni flýtigenanna *Ppd-H1*, *HvELF3*, *HvCEN* og *HvFT1*.

Samsetning samsætna þriggja marktækustu (marker-trait associations, MTAs) höfðu 214°dC lægri hitasummuþörf til að ná þroska, sem samsvarar 30 dögum á Íslandi. Nokkur genasæti fundust sem virðast gefa góða aðlögun að íslenskum aðstæðum hvað hitasummu varðar; m.a. *HvELF3*, *HvGA20ox2*, and *HvGA20ox1*.

Vetrargerð *Ppd-H1,* í tengslum við *HvFT1* samsætum, olli ýktum flýti í íslensku vorbyggi, en með mikilli minnkun á uppskeru. Vetrargerðar samsæta af *HvCEN* var einstök í íslenskum og finnskum byggyrkjum þar sem hún sameinaði mikinn flýti og háa uppskeru. Fyrri rannsóknir hafa fundið tíðnihalla í dreifingu *Ppd-H1* og *HvCEN* samsæta eftir breiddargráðu. Í arfgerðum sem aðlagaðar eru norðurslóðum sást önnur dreifing samsæta í *Ppd-H1* og *HvCEN* en sést hefur í evrópsku vorbyggi.

Þekking á samsætufjölbreytni tengdri aðlögun að lágu hitastigi á vaxtartíma eykur möguleika á því að auka gæði byggs og þar með möguleika á fjölbreyttari notkun á uppskerunni til dæmis til möltunar og til beinnar nýtingar til manneldis. Þekking á erfðum byggs með góða aðlögun að lágu hitastigi mun gera kynbótastarf markvissara við að velja kynbótalínur með góða aðlögun að norðlægum slóðum. Þetta mun færa framleiðslumörk byggs norður á bóginn, á Íslandi og víðar.

1. Introduction

1.1. Climate change effects on global food production

The human population is projected to keep growing for the next decades with a predicted peak at 10.9 billion people by 2100 (UN Department of Economic and Social Affairs - Population Division, 2021). With this increase, along with higher standards of living increasing demand for meat and dairy, the global agricultural system is already under sustained stress to deliver products of high-quality produce in ever increasing quantities (Dhankher & Foyer, 2018; Willett et al., 2019). This coupled to predictions of decreasing global crop yields under future climate conditions is a recipe for a perfect storm of food scarcity, undernourishment, and possible large-scale famines (Zandalinas et al., 2021). The previous methods of increased food production through breaking new agricultural land and/or increased fertilizer use are highly unlikely to shift the balance as little or no land is available for agriculture that has not been used already (FAO, 2018) and chemical fertilizers are already being used extensively, except perhaps in Africa.

Yields are in many cases already declining (Ray et al., 2019) with major grain producing regions already experiencing heat and drought stress caused by the changing climate which will only exacerbate negative impact on yields as temperatures continue to increase (Malhi et al., 2021). The ongoing biodiversity crisis makes it crucial to protect natural landscapes and ecosystems (Antonelli, 2022), making it necessary to sustainably intensify the production on agricultural land (Pretty et al., 2018). Soils are under increased pressure globally with increased erosion caused by unsustainable agricultural practices as well as altered precipitation patterns (Borrelli et al., 2020). All this combined can make the future look rather daunting. The most realistic way to increase output without increasing agricultural land or the use of fertilizers is using better adapted and more productive plant varieties.

The Arctic region is among the most rapidly transforming regions globally, since the temperature increase caused by global warming is drastically higher around the poles than in more equatorial latitudes (IPCC, 2022). Iceland and other Arctic and sub-Arctic regions could play an increasingly important role in the global food production system when these regions become more suitable for cereal production, while more traditional grain growing regions bear the brunt of negative aspects of the climate change. But while temperatures during the growing season may change, the photoperiod stays the same and therefore these regions will require cultivars specially developed for the unique conditions at high latitudes.

1.2. Climate in Iceland

Iceland, a large island located just south of the Arctic circle between 63° and 67°N, is an extreme environment for agriculture mainly for two reasons: temperature and daylength. The Gulf Stream and the North Atlantic Current carry warm waters from the Gulf of Mexico to the northern parts of the Atlantic Ocean, thereby creating a milder climate than on comparable latitudes elsewhere on the globe (Palter, 2015). The higher-than-expected sea temperatures make Iceland and parts of Scandinavia the world's most northerly agricultural regions. In the far north, the bright summer nights create extreme long-day conditions which can interfere with developmental processes in the plants, such as flowering, requiring plant breeders to develop locally adapted cultivars which flower at the right time to maximize the growth season (Mølmann et al., 2021).

Iceland is heavily influenced by the surrounding sea giving it a maritime climate. It has a relatively milder climate during winter than comparable latitudes in Scandinavia, but a cooler climate during the summer months. Average annual temperature in Reykjavik in the south-west is 5.4°C (2000 – 2020), peaking at 11.8°C during the warmest month (July) and dropping to 0.9°C during the coldest

month (January) (Icelandic Meteorological Office, 2021). In Akureyri in the north, the average annual temperature is 4.4°C (2000 – 2020), with the warmest month being July with 11.4°C, and February was the coldest month with -0.5°C. Annual precipitation in Reykjavik is 892 mm (2000 – 2020), with September as the wettest month with 98 mm and June being the driest month with 45 mm. Generally, April to August are the driest months, and September to March are getting the bulk of the precipitation (Icelandic Meteorological Office, 2021). In Akureyri, annual precipitation is 593 mm (2000 – 2020), with the most precipitation in December (80 mm), and the least in June (20 mm). The day length May 1st is approximately 17 hours in Reykjavik and Akureyri, increasing to 21 and 23.5 hours in Reykjavik and Akureyri, respectively, at summer solstice. September 1st, the day length has decreased to approximately 12.5 hours in Reykjavik and Akureyri.

The heat sum (°dC) is the sum of the average daily temperature (above a base line temperature) for a specified number of days, e.g., from sowing to maturity (Ruselle et al. 1984). A baseline temperature is used to capture the temperature range where there is physiological activity in the plant. For barley, often a baseline temperature of 5°C is used (Martin et al., 2017). A base line temperature of 0°C was used in this project as it gives the best estimator of development in Nordic conditions (Hermannsson, 1993; Strand, 1987). This gives a more precise estimator than number of days which will vary more depending on the climatic conditions at the location. The growing season is relatively long in Iceland with sowing usually possible in the latter half of April and harvest in September (Hilmarsson et al., 2017). However, because of its maritime climate, the accumulated heat sum during the growing season is lower in Iceland than other regions at comparable latitudes (Martin et al., 2017) especially during the grain-filling period in late summer and early fall. Martin et al. (2017) estimated cropping season degree days (with 5°C baseline temperature) for locations in Norway, Faroe Islands, Orkney, Shetland Islands, Iceland, Greenland, and Newfoundland. All but one location, had a higher heat sum in the growing season than Iceland, and even the most northerly location in their study (Alta, Norway, 69°58'N) had a heat sum of 729, whereas Akureyri, and Reykjavik in Iceland had 555 and 590°dC, respectively (a heat sum 20 – 24% lower than Alta). Only Narsarsuaq in Greenland had a lower heat sum with 474°dC.

1.3. Barley: Importance, origin, and domestication

Barley is the fourth most important cereal globally, after wheat, maize, and rice, both in production value and area cultivated (FAO, 2021). It is generally more tolerant to marginal areas such as dry land, high altitudes, and high latitudes, than wheat. In the Nordic region, barley is the most important cereal crop in four out of five countries, with average yields in 2019 ranging from 3.6 in Iceland to 6.2 t ha^{-1} in Denmark (FAO, 2021). Despite its adaptability, grain yield in the more northern regions still trails behind more favourable areas. The yield gap will be even more important to bridge when effects of climate change become more severe. Breeding of cultivars better adapted to local environments could potentially close the yield gap in northern latitudes. In Iceland, this would translate into a more self-sufficient food and feed production with less dependence on imports, which is projected to be increasingly important as the climate change effects become more severe. Barley is used primarily for feed and malt for the brewing industry (Langridge, 2018). A higher quality of the grain, which is obtained in filled and mature barley, will enable more diversification in the produce for human consumption. Barley is also a model crop for cereal research; with its diploid genome and inbreeding habit it is well suited as a model species for closely related cereal species with more complex genomes such as wheat (Schmid et al., 2018). It has been extensively studied globally, not least with respect to flowering time, but prior to the initiation of this project no knowledge of the molecular genetic diversity existed for the Icelandic barley cultivars

and breeding lines. Since then, two B.Sc. studies (Bos, 2016; Sigurðardóttir, 2019) and one M.Sc. study (Bos, 2019) which focused on earliness genes (*HvFT1*, *Ppd-H1*, and QTL mapping), have been published.

Barley was one of the very first crops to be domesticated by early Neolithic farmers in the Fertile Crescent some 10,000 years ago (Zohary et al., 2012). The wild progenitor, *H. vulgare* ssp. *spontaneum* (C. Koch) Thell., is still found in the centre of domestication. Domestication genes such as the non-brittle genes ('*btr1*' and '*btr2*') were among the first to be selected for (von Bothmer & Komatsuda, 2010), followed by spike morphology genes ('*vrs1*', producing a six rowed inflorescence rather than the wild-type two rowed type), and seed dormancy ('*SD1*' and '*SD2*') (Civáň et al., 2021). Wild barley is a long day responsive plant that flowers when day length exceeds a critical length but shows a substantial delay in flowering when grown under short day conditions (Laurie et al., 1995), although, day length neutral plants, found among both wild barley and as induced mutations have also been identified and genetically characterized (Gustafsson et al., 1971). In its centre of origin, barley germinates in the fall and stays in the vegetative phase during the cool and humid winter season. In the spring, increased day length triggers the onset of flowering, and the plants mature at the start of the dry summer period ensuring a period of dormancy for the seeds during the hot and dry summer (Lister et al., 2009).

Over the millennia, barley spread with farmers northwards, and is estimated to have reached southern Scandinavia around 6,000 years ago (Sørensen & Karg, 2014). The move northwards required an adaptation to the longer day length and an altered agronomic practice, where the barley was sown in spring instead of fall, as was practiced in the centre of origin and in the southern parts of Europe. Barley was cultivated in Iceland by the first settlers in the 9th century and was grown continuously for around 400 years, until the climate worsened in the Little Ice Age (Hermannsson, 1993). Sporadic cultivation may have occurred during the centuries that followed. However, it was first in the early 20th century that barley cultivation was again tried on a larger scale. Cross breeding and variety testing of barley was started in 1976 at The Agricultural Research Institute (RALA) in Reykjavik and this laid the foundation of the increasing barley cultivation seen in Iceland the last 30 years (Sigurbjörnsson, 2014; Hilmarsson et al., 2017). In the southern part of the Nordic region primarily two rowed (2r) spring barley is grown for malt production, and in the northern part, including Iceland, both 2r and six rowed (6r) barley is grown primarily for feed production.

1.4. Traits of importance - Genetics of adaptive traits

Research by a joint Nordic consortium for the promotion of plant breeding (Samnordisk planteforædling, 1992) showed that temperature is the climatic factor that mostly affects plant growth in northern Scandinavia. Early flowering and the ability to reach maturity at low temperatures are therefore key components to secure a high and stable yield at high latitudes (Hilmarsson et al., 2017; Nurminiemi et al., 1996). In the cold temperature Icelandic growth season the barley grains typically are harvested at a high moisture content. This makes storage difficult as humid seed will mould. Icelandic barley farmers typically need to dry the grains after harvest, from approximately 40 – 50 % moisture content to around 15%. The drying is energy consuming and thereby an added cost. In addition, the process of grain filling is often incomplete, rendering a lower quality of the grain. Breeding of barley which can produce mature grain in low temperature will thus save money and could improve the quality of the yield.

Additionally, it is important to keep in mind that events of strong winds and heavy precipitation are likely to increase in frequency due to climate change (Coumou & Rahmstorf, 2012) and therefore exacerbate the already negative effects of the Icelandic weather on yield stability in Iceland. Hence, resistance to lodging and straw breaking are important traits in addition to the correct timing of flowering. Tall plants are prone to lodging after strong winds accompanied by heavy rainfall, thus leading to yield losses (Dockter & Hansson, 2015).

1.4.1. Flowering time

Timing of flowering, through seasonal cues, such as day length and temperature, is a key element for reproductive success and high yield (Andrés & Coupland, 2012; Fernández-Calleja et al., 2021; Wiegmann et al., 2019). Earliness is a complex trait where genetic variation can greatly alter the plants' response to day length and temperature (Blümel et al., 2015; Cockram et al., 2007). Consistent with its importance for the plants' survival the response to changes in the day length is controlled by a network of well conserved genes (Blümel et al., 2015). Flowering time genes are generally divided into three groups: vernalization genes (e.g. *VRN-H1*, *VRN-H2* and *HvFT1 (VRN-H3*)), photoperiod genes (e.g. *Ppd-H1* and *Ppd-H2*), and *earliness per se* (*eps*) genes controlling flowering independently from day length or temperature (e.g. *HvGA20ox2* and *HvCEN*) (Wiegmann et al., 2019).

1.4.2. Vernalization response

Vernalization is defined as "the acquisition or acceleration of the ability to flower by a chilling treatment" (Chouard, 1960). Barley has three major vernalization genes that are responsive to a period of low temperature: *VRN-H1* on chromosome 5HL (Trevaskis et al., 2003), *VRN-H2* on 4HL (Laurie et al., 1995; Yan et al., 2006), and *HvFT1 (Vrn-H3*) on 7HS (Faure et al., 2007). *VRN-H1* is stimulated by vernalization and speeds up the shift from vegetative to reproductive development at the shoot apex (Trevaskis et al., 2007). *HvFT1* is induced by a long photoperiod and further speeds up the reproductive apex development (Trevaskis et al., 2007). *VRN-H2* is a floral repressor which act as a repressor of *HvFT1* until the vernalization requirement is fulfilled (Trevaskis et al., 2007). Winter barley has an active *VRN-H2* allele, whereas spring barley lacks the *VRN-H2* locus (Trevaskis et al., 2003). In winter barley, after a period of vernalization, the wild-type recessive allele of *VRN-H1* promotes a shift to the reproductive phase by inducing the development of the reproductive meristem; *HvFT1* is thought to speed up the late developmental stages, and *VRN-H2* represses *HvFT1* until the plant has vernalized (Distelfeld et al., 2009). In spring barley, the dominant allele of *VRN-H1* does not require vernalization, and the *VRN-H2* gene is deleted, leaving photoperiod as the main cue to determine flowering time. *HvFT1* is induced by long days, and an activation of the florigen production leads to an acceleration of the reproductive apex development. Wiegmann et al. (2019) identified polymorphisms in the intron of *VRN-H1* that could differentiate between winter and spring barleys. Although winter and spring barley are the two main types, the difference is not clear cut. Facultative barley tolerates vernalization but will flower without it. Barley with a facultative growth habit has lost the functional *VRN-H2* but has the *VRN-H1* locus with the full intact intron 1 region (Muñoz-Amatriaín et al., 2020). This means facultative barley can be sown in the fall and has a good frost tolerance but can also be sown in the spring without need for vernalization. *HvFT1* has alleles with copy number variation (CNV) which have been associated with early flowering in spring barley, a phenotype first discovered in the Finnish cultivar 'Tammi' (Loscos et al., 2014; Nitcher et al., 2013). *HvFT1* has a role in regulating the expression of florigen which is a signal for the

plant to initiate flowering. Introgression of the 'Tammi'-allele in winter background overrides vernalization requirement, as shown by Nichter et al (2013) and Cuesta-Marcos et al. (2015).

1.4.3. Photoperiod response

Ppd-H1 was described by Turner et al. (2005) as an orthologue of the *Arabidopsis* pseudo response regulator gene *PRR7*. Natural variation in the locus *Ppd-H1* is believed to have facilitated the shift from a strictly long day responsive plant to the less photoperiod sensitive barley now cultivated at higher latitudes (Jones et al., 2008; Lister et al., 2009). The allele frequency shows a latitudinal gradient where the dominant day length responsive *Ppd-H1* is present in south Europe (Lister et al., 2009) whereas a naturally segregating/occurring allele, *ppd-H1*, found for example in wild barley in the Zagros mountains in Iran, has been found to have a much higher frequency in cultivated barley from northern latitudes and provides non-responsiveness to long days (Morrell & Clegg, 2007; Schmid et al. 2018). This enables the spring sown barley to develop vegetatively before onset of flowering which helps to produce a high yield (Sharma et al., 2020). In northern latitudes (>60˚ N), spring sown barley is the predominant cereal crop.

1.4.4. Earliness per se

In addition to the importance of *Ppd-H1*, another locus important for the northward expansion of barley is *HvCEN*. Comadran et al. (2012) highlighted the contribution of allelic variation at *HvCEN* as a factor to enable latitudinal range extension of barley and, recently, Bustos-Korts et al. (2019) and Jayakodi et al. (2020) reported variation at the *HvCEN* locus as contributing to early flowering. A resequencing study of *HvCEN* identified haplotypes in a collection of wild and cultivated genotypes, where they showed a higher proportion of early flowering alleles in cultivated genotypes (Casas et al., 2021). Furthermore, it showed that the early flowering alleles were also present in wild genotypes (in lower frequencies), confirming that domestication relied, at least to some extent, on existing allelic variation. ‘Haplotype III’ found in Casas et al. (2021) is found in wild barley and fixed in northern European spring barley cultivars, whereas ‘haplotype II’, also common in wild barley, is found in winter barley cultivars. This indicates a strong adaptive selection pressure at the *HvCEN* locus (Casas et al. 2021). *HvCEN* has been proposed to be a paralog to *HvFT1* (Casas et al. 2021).

A specific mutation found in the *HvELF3* gene has been proposed as enabling barley cultivation in northern Scandinavia and Iceland (Zakhrabekova et al., 2012). Spring barley carrying this allele flower earlier in long-day conditions than the mother cultivar to the induced mutation. Xia et al. (2017) identified wild barley from Tibet that carried day-length neutral inducing alleles of *HvELF3* whereas the widely used *HvELF3* allele that is most frequent in Nordic barley was derived from the induced mutant cv. ‘Mari’ developed in 1960 (Dormling et al. 1966).

It is known that the early flowering of some barley genotypes is closely linked to gibberellin biosynthesis (Boden et al., 2014). He et al. (2019) reported that gibberellin is an important signal in flower development in barley. Kupke et al. (2022) demonstrated the role of gibberellic acid (GA) in barley development by exogenous application of a GA inhibitor, causing a delay in flowering for up to 200 °dC.

1.4.5. Height

Plant height is an important trait for adapting barley to windy and rainy environments since short and strong stems help to prevent lodging in addition to positively affecting the harvest index (Hay, 1995). The semi-dwarfing genes have been an important component of the plant breeder’s toolbox

ever since the Green Revolution, when they were utilized to breed new cultivars of wheat and rice which could withstand higher fertilizer applications without lodging (Peng et al., 1999), thus dramatically increasing yield. Alleles responsible for the Green Revolution have been identified in the reduced height gene (*rht1* and *rht2*) in wheat, and the semi-dwarf gene (*sd1*) in rice (Dockter & Hansson, 2015). In barley, the semi-dwarf locus (*sdw1/denso*) was identified as an ortholog of the rice *sd1* gene (Jia et al., 2009), later identified as the *HvGA20ox2* gene (Xu et al., 2017). The *HvGA20ox2* gene has several known alleles where three were induced by mutagenesis: *sdw1.a* was induced in the cv. 'Jotun'; *sdw1.d* was induced in cv. 'Valticky', and released as cv. 'Diamant'; *sdw1.e* was induced in cv. 'Bomi' and released as 'Risø no. 9265'; and finally *sdw1.c* was discovered in cv. 'Abed Denso' (Jia et al., 2009). Allelic variants of semi-dwarfing genes have been widely employed in modern barley breeding (Kuczyńska et al., 2013). Modern European barley cultivars generally depend on allelic variation in the *HvGA20ox2* gene as their source of semi-dwarfing (Kuczyńska et al., 2013). In addition to reduced height, *HvGA20ox2* alleles have been reported to cause delayed heading. Kuczyńska et al. (2013) reported a 10- to 20-cm height reduction in *sdw1* plants, relative to wild type plants.

Barley has three *HvGA20ox* genes: *HvGA20ox1, HvGA20ox2*, and *HvGa20ox3* (Xu et al. 2017). The partial or total loss of function of the *HvGA20ox2* gene could be compensated by enhanced expression of its paralogs *HvGA20ox1* and *HvGA20ox3* (Xu et al. 2017). Spielmeyer et al. (2004) mapped *HvGA20ox1* to chromosome 5H, and *HvGA20ox3* to chromosome 3H.

Another semi-dwarfing gene is the *breviarestatum-e* (*ari-e*) which has been fine-mapped to around 488 Mbp region on chromosome 5H (Jia et al., 2016). Wendt et al. (2016) proposed the *Dense and Erect Panicle 1 (DEP1)* as the candidate genes underlying the *ari-e* locus. Varieties carrying the *ari-e* mutation have a semi-dwarf phenotype (Dang et al., 2020).

1.5. Genomics of barley and the tools for exploring and exploiting diversity

The size of the barley genome is estimated to 5.1 Gbp (Dolezelt et al., 1998). Since the first whole genome sequencing project was published (The International Barley Genome Sequencing Consortium, 2012) also a pan-genome has been published (Jayakodi et al., 2020). These collaborative efforts have paved the way towards a better knowledge on barley diversity. Barley has a large genome and consists of approximately 80% repetitive sequences (Rajendran et al., 2022) with a high linkage disequilibrium in cultivated barley. This makes whole genome sequencing challenging, however restriction enzyme-based protocols for genotyping-by-sequencing (GBS) have proven useful and can provide a large number of SNPs that can further be analysed in GWAS or QTL analyses. Besides GBS, for barley, two SNP chips are commonly used: the 9K iSelect Illumina Infinium array (Comadran et al., 2012) that contains 7,842 gene-based SNPs of which 6,094 SNPs have known physical positions, and the 50K Illumina Infinium iSelect genotyping array containing 44,040 SNPs (Bayer et al., 2017).

Marker assisted selection (MAS) has long been used by breeders to speed up and bring precision to the selection process (Collard & Mackill, 2008). Qualitative traits, such as many disease resistance genes, are particularly suitable for MAS as they require only one allele to be present/absent to obtain the desired trait. Quantitative traits are also suitable for MAS provided there is sufficient knowledge on the genetics behind the trait to facilitate a pyramiding of the necessary alleles in the desired genetic background.

1.6. Genome wide association studies

Genome wide association studies (GWAS) have become standard practice in genetic studies of crops alongside the development of crop specific high throughput SNP platforms, see e.g. Alqudah et al., 2020). One main advantage compared with QTL mapping is that GWAS can handle diverse populations with no need to perform crossings. For the analysis to work, the phenotypic variation needs to have a normal distribution without extreme outliers, and the traits should have a high broad sense heritability. The size of the population is critical and should typically be in the range of 100 – 500 individuals (Alqudah et al., 2020). Smaller population sizes risk reducing the power of the GWAS. One drawback from using diverse unrelated (or distantly related) populations is that they often are structured, e.g., by row type or geographic origin. If such population structure is not accounted for in the model, this can lead to spurious associations. The allele frequency needs to be above 5% for the associations to be detected in the GWAS, thus, rare alleles will be filtered in the initial phase and remain undetected. Linkage disequilibrium (LD) will affect the analysis. Barley is an inbreeding species with a high level of LD compared to outbreeding species like maize. This means in practice that fewer markers are needed in barley, but also, that the resolution is lower caused by LD creating genomic regions with low level of recombination. To navigate the pitfalls of GWAS model selection is key. Models are validated based on heritability estimates and quantile-quantile (QQ) plots. They either take no consideration of the population structure, e.g. general linear model (GLM), or they use information from kinship analysis, principal component analysis (PCA), or Q-values from STRUCTURE, in various kinds of mixed linear models (MLM). To account for population structure, three approaches are commonly used: Kinship (van Raden), PCA, and STRUCTURE analysis.

A threshold value for the p-values is needed to evaluate the marker trait associations. The threshold can be based on the Bonferroni correction, where a significance level is divided by the number of SNP markers, resulting in a standardized threshold for all traits (Storey & Tibshirani, 2003). Bonferroni correction is generally considered a strict threshold with the risk of missing biologically meaningful associations. A suggestive threshold, earlier published by Duggal et al. (2008), allowing for one false positive per genome scan can be estimated by dividing 1 by the number of markers for each panel. A third approach is to use a standardized value of -log(p) = 3 (Li et al. 2012). Another approach is the false discovery rate (FDR), where threshold values for significance are calculated trait wise (Storey & Tibshirani, 2003). FDR is calculated for each trait independently, which makes it more powerful in studying the genetic factors of developmental and agronomic traits in crop plants. FDR has been widely used and is a flexible approach that take into consideration differences in accuracy between the traits studied.

1.7 Utilizing genetic diversity in the barley genepool

Cultivated barley is a domesticated form of the wild barley (*Hordeum vulgare* ssp. *spontaneum*). There is no crossing barrier between the wild barley and cultivated barley, meaning the wild species in the primary gene pool are available when searching for beneficial alleles to introgress into elite germplasm. One secondary gene pool is available, *Hordeum bulbosum*, which can be crossed with difficulty into cultivated barley. The other around 30 species of the genus *Hordeum* are unavailable to use for breeding of barley using traditional crossings (Mascher et al., 2018).

But barley breeders only rarely go all the way to the wild species, as this involves a large portion of linkage drag and requires many back crosses to eliminate all other undesirable genes that follow. The main source of allelic diversity for use in barley breeding is found in landraces that have been conserved in gene banks around the world. In total, there are around 485.000 accessions of the

genus *Hordeum* in genebanks, out of which around 300.000 are the cultivated form of barley (cultivars new and old, and landraces) (Knüppfer, 2009).

The first step in utilizing non-elite germplasm in a breeding program is pre-breeding. This involves searching for a likely allele donor (older or exotic cultivars, landraces, or accessions of the wild barley). After screening of potential donors for the trait of interest, a scheme of crossings is made to introgress the allele of interest into elite germplasm without too much linkage drag. Once this has been accomplished, traditional cross breeding between elite material can continue.

The stage of pre-breeding is resource demanding and can be challenging for the rather small companies and entities (mainly private but also public) that are active in the Nordic region. As such, pre-breeding is especially suitable for precompetitive collaboration, with the help of public funding.

Such a program was launched in 2011 by the Nordic Council of Ministers and was called the Nordic Public-Private Partnership (PPP) for pre-breeding. Six Nordic barley breeding companies/institutions were part of the joint public-private partnership (PPP) for pre-breeding in spring barley (Bengtsson et al., 2017). The partners participating as plant breeding entities in the PPP barley project were Nordic Seed (DK), Sejet (DK), Lantmännen (SE), Boreal (FI), Graminor (NO), and the Agricultural University of Iceland (AUI) (IS). Copenhagen University, Natural Resources Institute Finland and the Swedish University of Agricultural Sciences participated as academic partners along with the Agricultural University of Iceland.

2.The Thesis

2.1. Objectives

This thesis presents the genetic diversity of Icelandic barley in the context of the current Nordic advanced barley breeding gene pool (Paper I), and against a subset of specifically chosen early Nordic barley cultivars and breeding lines (Paper II). Furthermore, detailed knowledge on allelic diversity in specific earliness loci was obtained (Paper III). The results can be directly utilized for a focused breeding of early maturing barley with simple means of molecular markers and pyramiding of desired alleles.

Prior to this project no molecular knowledge of the Icelandic barley cultivars and breeding lines existed, although harvest results had conclusively demonstrated their unique behaviour under Icelandic environmental conditions. The initial aim was therefore to characterize the material at the molecular level with an emphasis on genes/markers related to traits identified as important for Icelandic conditions, such as flowering time, early maturity, and height. The small-scale breeding program has been carried out by traditional crossings followed by field scorings and selections without the aid of molecular tools. Modern molecular tools such as marker assisted selection, genomic selection, and gene editing, could substantially speed up the process and add precision to the selection work and assist in selecting the parental lines for new crosses. These technologies require knowledge of what genes, alleles, and allele combinations are favourable for the breeding targets. This will be even more important in a rapidly changing climate.

Besides yield, improved earliness has been the main objective of the Icelandic barley breeding program. Several breeding lines have been developed which show “extreme earliness”. The advantage of early maturing lines is that the dry matter content upon harvest is higher. One

drawback from the low temperature in Iceland is that the barley seed has in general a relatively high humidity when harvested. By developing early maturing cultivars, the grain will be dryer upon harvest and will require less post-harvest drying. Another advantage of early maturing barley is that it can be harvested earlier. In Iceland, autumn storms are common and have the potential to severely reduce the harvest. A shorter time in the field, especially in the autumn, will lower the risk of crop failure due to severe weather.

With this as a background, the objectives of the thesis were to:

- Test the earliest Icelandic breeding lines in multi-environment field trials in the Nordic region, and beyond, in the context of the current Nordic barley elite gene pool.
- Test the Icelandic genotypes together with Nordic material in controlled environments, with emphasis on day length and temperature.
- Use genome-wide association to link traits, including heading, maturity, and height, to markers and putative loci.
- Study allelic diversity at selected loci by resequencing.
- Find allele combinations of adaptive importance for a northward expansion of barley cultivation.
- Trace the origin of adaptive alleles through resequencing and pedigree analysis.

3. Material and Methods

3.1. Plant material

3.1.1. PPP panel (Paper I)

In the first phase of the Nordic PPP pre-breeding project, each of the six barley breeding entities involved selected 30 genotypes to represent the current diversity in each breeding program with regards to pathogen resistance, and agronomic traits such as earliness and straw quality among advanced cultivars and breeding lines. This resulted in a panel of 180 genotypes representing the advanced Nordic breeding gene pool of spring barley. The PPP panel consisted of 130 2r and 50 6r genotypes. The 6r genotypes came from Icelandic, Norwegian, and Swedish partners whereas the 2r genotypes came from all six participants. It should be noted that the partner from Finland decided to exclude their 6r genotypes, which where thereby lacking in the PPP panel. The panel was grown in field trials across the Nordic region, ranging from Iceland to Denmark, and with an additional trial in south Germany (Figure 3).

It was concluded, after genotyping the PPP panel, that 169 genotypes were unique, whereas eleven genotypes were duplicates added to the project by more than one entity. Out of the 169 genotypes 124 were 2r and 45 were 6r. 58 lines were of Danish origin (all 2r), 30 Swedish (28 2r and 2 6r), 30 Norwegian (3 2r and 27 6r), 29 Finnish (all 2r), 21 Icelandic (5 2r and 16 6r), and one from the United Kingdom (2r). For parts of the analyses, the whole panel (PPP169) was subdivided into only the 2r lines (PPP124).

3.1.2. Greenhouse panel (Paper II)

Twenty-seven 2r and 57 6r spring barley cultivars and breeding lines of northern European origin were selected for analysis. The lines were selected with the aim of representing diversity in heat sum requirements to maturity and day-length responses. The selection was based on previous results from performance in field trials (Nurminiemi et al., 1996), experience from the Icelandic barley breeding program (J. Hermansson and M. Göransson, unpublished results) and the results of a preliminary greenhouse study (M. Lillemo, unpublished results). Three near-isogenic lines (NILs) with allelic diversity at the *HvELF3* and *Uzu* loci were included along with their two-rowed mother cultivar Bowman to compare the phenotypes of known mutant lines with the Nordic population.

3.1.3. Resequencing panel (Paper III)

The resequencing panel was based on previous knowledge of the earliest lines from field trials in Iceland and from the PPP project. The lines included two extremely early breeding lines ‘247-11’ and ‘247-1’ (both low yielding), and the early and high yielding breeding lines ‘292-2’, ‘292-51’, and ‘06-130’. With the help of logbooks, and online information from genebanks, as well as publications (Manninen & Nissilä, 1997; Nurminiemi et al., 1996), pedigrees were constructed, and parental lines were selected for inclusion in the panel. In addition to the early breeding lines and selected parental lines, a few high yielding Nordic cultivars were included for reference.

Twenty barley genotypes were selected by analysing data from geographically dispersed Icelandic cultivar trials between the years 1987 and 2014 (Hilmarsson et al., 2017). The selection was made to include (1) previously known extremely early lines (‘247-11’ and ‘247-1’) (Paper I & II), (2) selected ancestors based on pedigree data, and (3) cultivars and breeding lines with a high yielding capacity

in Iceland. The panel included Icelandic cultivars and breeding lines as well as foreign cultivars that had been tried in a minimum of four trials with yield data.

The three panels overlap partly as seen in Figure 1.

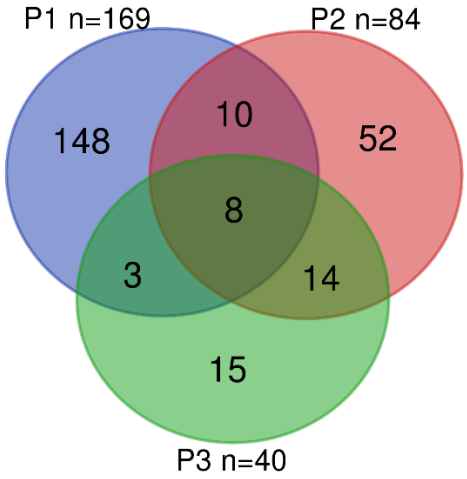

Figure 1: Venn diagram showing overlap among genotypes in the three study panels in Papers I-III (P1 – P3).

3.2. Phenotyping

In the PPP panel heading day was recorded when half the spike was visible in 50% of the plants in each plot, corresponding to Zadoks growth stage 53 (Zadoks et al. 1974), illustrated as B in Figure 2. Maturity was registered as when the peduncle turned yellow in 50% of the plants in the plot, corresponding to Zadoks growth stage 87 (Zadoks et al. 1974). The heat sums were calculated from sowing day until heading and maturity, respectively. The heat sum to heading and maturity were subsequentially used in GWAS analyses. Height was measured as the average height from soil level to below the spike in each plot (corresponding to the straw length).

In the greenhouse panel, heading day was registered as the day of ~2 cm of awn emergence of the second earliest plant in each pot, corresponding to Zadoks growth stage 49 (Zadoks et al., 1974), illustrated as A in Figure 2. This is the growth stage best corresponding to the actual fertilization event in spring barley (Alqudah & Schnurbusch, 2017). Maturity was recorded when the peduncle of the earliest straw of the second earliest plant in each pot turned yellow, indicating that the phloem ceased to allocate sugars to the kernels, corresponding approximately to Zadoks growth stage 87 (Zadoks et al., 1974). Heat sums from sowing until heading and maturity, respectively, were calculated and used in subsequent analyses. Height was registered as straw length from soil level to below the spike. The experiment was divided into four pre-set temperature and day length regimes (Figure 4): long day warm (LDW) with 20 hours of day light and 15°C (night) and 25°C (day) temperature, averaging 20°C; long day cold (LDC) with 20 hours of day light and 5°C (night) and 15°C (day) temperature averaging 10°C; short day warm (SDW) with 12 hours of day light and 15°C (night) and 25°C (day) temperature; short day cold (SDC) with 12 hours day length and 5°C (night) and 15°C (day) temperature. The actual recorded temperatures deviated slightly from the pre-set temperature and were used in the heat sum calculations.

In the resequencing panel, the traits were yield (t ha^{-1}) and heading (number of days from sowing to heading). The heading was scored when half of the plants in the plot had reached Zadoks growth stage 55 (Zadoks et al., 1974), illustrated as C in Figure 2.

The discrepancy between measurements of heading day between the three experiments reflects the confusion that led up to the publication by Alqudah and Schnurbusch (2017), where the awn protruding stage (Zadoks stage 49 (Zadoks et al. 1974)) was determined as best corresponding to the actual fertilization event.

Figure 2: Stages of heading from Alqudah and Schnurbusch 2017.The three stages are illustrated as (A) awn tipping, (B) half-spike, and (C) full spike.

Heat sums were calculated from sowing to heading and from sowing to maturity in both Papers I and II.

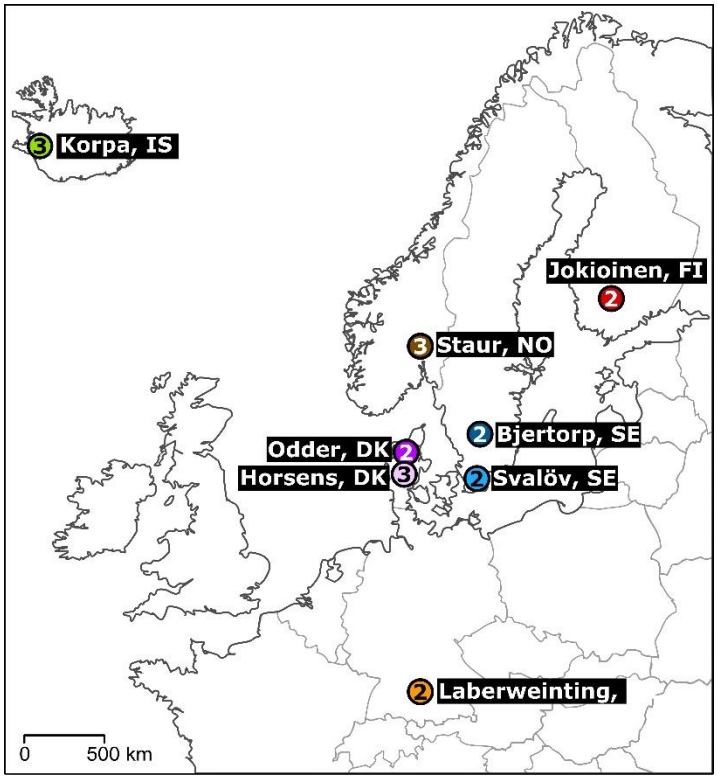

Figure 3: Field trials carried out in paper I. Numbers in circles next to the location names indicate number of years.

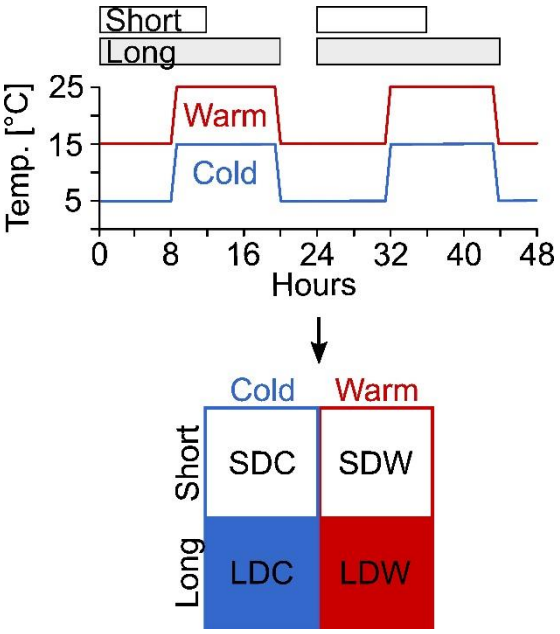

Figure 4: The four environments in the controlled experiment reported in Paper II. Short day cold (SDC); short day warm (SDW); long day cold (LDC), and long day warm (LDW).

4. Results and discussion

In the following the main results of this thesis research will be discussed in relation to the research objectives:

4.1. Test the earliest Icelandic breeding lines in multi-environment field trials in the whole Nordic region, and beyond, in the context of the current Nordic barley elite gene pool

In the PPP panel, three Icelandic breeding lines ('247-11', '247-1', and 'Hrutur') were the earliest heading in all environments and years, a total of 19 field trials in climatic conditions ranging from south Germany up to Iceland (Figure 3). This is a clear result that the earliness in Icelandic genotypes is unique in a Nordic context. We attribute this to the local breeding programme which have actively done field selections for early genotypes.

In Scandinavia, summer temperatures are higher than in Iceland, giving the barley a better chance to reach maturity. In Iceland, the highest temperature of 11.8 degrees is reached in July and August. The cool summer is a strong selective pressure for early maturing genotypes and can explain the strong effect of the local selection. In Scandinavia, the extremely early heading genotypes are easily outyielded by later maturing genotypes.

In a wider context, early heading and maturing barley can be beneficial in other marginal regions such as dry areas and areas where summer temperatures can induce heat stress. The consistent results from field trials as far south as Germany show that the earliness is consistent in a variety of environments, and the genotypes merit further studies in other climatic conditions.

4.2. Test the Icelandic genotypes together with Nordic material in controlled environments, with emphasis on day length and temperature

The results from field trials (**Paper I**) were followed up by an experiment in controlled conditions (**Paper II**). Here, we could see that the same three genotypes ('247-11', '247-1', and 'Hrutur') were indeed earliest heading in long day environments (20-hour day length), irrespective of temperature. However, in short day conditions (12-hour day length) they were surpassed by several day length neutral genotypes which headed earlier.

We identified Nordic day length neutral genotypes, and these were confirmed by their pedigree to derive back to the Swedish cv. 'Mari'. The specific mutation in *ELF3* which gives the day length neutral character of 'Mari' is an induced mutation and was described by Dormling et al. (1966) and Gustafsson et al. (1971). All day length neutral lines in the experiment had 'Mari' in their pedigree.

Kim et al. (2022) studied barley height in four different regions in South Korea and found that the warmest region produced the tallest plants. In Paper II, this pattern was reversed during long day conditions in the controlled environment trial. Most genotypes grew taller in cold temperature. 'Bowman NIL Uzu1', 'JO1279', and cv. 'Edel' showed the highest variability in height. However, we identified genotypes that were stable in height: 'Tampar', 'Teista', and 'IGP-M-268' were promising candidates to use in further studies focusing on height stability. Interestingly, the genotypes with weakest height response, and hence highest height stability to temperature were all selected in a cold environment. Height stability is of importance in cold and windy environments like Iceland where imported cultivars which respond to the lacking summer heat by growing taller, are more prone to lodging which can result in yield losses.

4.3. Use GWAS to find marker trait associations, and putative loci, for heading, maturity, and height

Heading

The main findings from **Paper I** were associations with heat sum to heading where MTAs at *HvGA20ox2* (*denso/sdw1*), and *HvCry1b* were found in the PPP169 panel. In the PPP124 panel, in addition to *HvGA20ox2 HvCry1b*, also *HvTOC1* were found.

Earlier studies have identified *HvGA20ox2* as associated with delayed heading in barley (Jia et al. 2009). We could see that the locus influences the heading day in **Paper I** and found the late heading genotypes to be shorter in height, indicating a functional allele of *HvGA20ox2*. *HvCry1b* is a blue light receptor gene, involved in the regulation of the abscisic acid (ABA) pathway leading to an increased seed dormancy in blue light exposed barley seed (Barrero et al., 2014). In dicots, *Cry1* is involved in other aspects of plant development than germination, such as hypocotyle elongation, and leaflet expansion. Monocots have two copies of *Cry1* (*Cry1a* and *Cry1b*) with overlapping functions. It is believed that the less knowledge of the function of *Cry1* genes in monocots is because of the difficulties in isolating mutant genotypes with one of the *Cry1* genes isolated (Barrero et al. 2014), however reports on effect of blue light on coleoptile elongation have been reportedly coregulated by *Cry1* and *Cry2* genes in barley (Barrero et al. 2014). *Cry1* and *Cry2* genes have also reportedly a function i root and shoot development in barley and is upregulated by increased day length (Schneider et al., 2022). *TOC1* is a part of the circadian clock in *Arabidopsis* (Müller et al., 2020). Attempts have been made to place *HvTOC1* in the barley circadian clock but so far the role is unclear (Müller et al., 2020).

In **Paper II**, the main MTA for heat sum to heading which was shown in all environments was *HvELF3*. This is in line with an earlier study by Zakhrabekova et al. (2012) which found that *HvELF3* and specifically the allele derived from cv. 'Mari' contributed to the northward expansion of barley. This was however not supported by results in **Paper III**, see section 1. 4. below.

Maturity

We found *HvGA20ox2* (*denso/sdw1*), *HvCry1b*, and *HvKAO1* as the most significant MTAs for heat sum to maturity (**Paper I**). *HvCry1b* is a CHRYPTOCHROME locus, which is regulated by blue light and has previously been reported to stabilize the CONSTANS protein in *Arabidopsis* (Gawroński et al., 2014). *HvCO1* and *HvCO2*, the barley orthologs to *Arabidopsis CONSTANS*, are involved in the barley flowering pathway (Mulki & von Korff, 2016). The specific role of *HvCry1b* is yet to be described. *HvKAO1* is reportedly involved in the GA biosynthesis in barley (Spielmeyer et al., 2004) and in *Arabidopsis* (Regnault et al., 2014), so it involves the same pathway as *HvGA20ox2*.

In **Paper II**, we found the *Ppd-H1, DHAR2, HvFT1,* and *HvELF3* genes to be associated with heat sum to maturity. *Ppd-H1* was found in the same lines as in **Paper I** and could be detected in the GWAS analysis as the allele frequency was higher in the Greenhouse panel. *DHAR2* has previously been reported to have a role in salt and heat tolerance in *Arabidopsis* (Bulley et al., 2021) and drought tolerance in sorghum (Azzouz-Olden et al., 2020). *DHAR2* was only significant in the LDW treatment indicating that it might have a role in barley heat or drought stress tolerance. *HvFT1* is a gene well known in the flowering pathway (Faure et al., 2007), as well as *HvELF3* (Zakhrabekova et al., 2012; Faure et al., 2012) and we hereby establish their effect also in Nordic barley.

Height

In **Paper I**, the *HvGA20ox2* locus was associated with height. The GA20 oxidase gene (*Hv20ox2*), involved in biosynthesis of gibberellic acid (GA), has been identified as a candidate for the *denso/sdw1* gene (Jia et al., 2009). The *HvGA20ox2* has been widely utilized in European barley breeding (Jia et al., 2009; Kuczynska et al., 2013; Dockter and Hansson, 2015) and it was an expected finding in the Nordic barley breeding gene pool. Another association was found for height, the *HvDWF11* locus (Dockter et al., 2014), which has earlier been reported as a brassinosteroid-related gene in rice (Tanabe et al., 2005) and in *Arabidopsis* (Locascio et al., 2014).

In **Paper II**, the main association for height was with markers 0.3 Mbp from the *HvGA20ox1* locus. The *HvGA20ox1* gene is a homologue to *HvGA20ox2*, which has been reported to compensate for reduced function of *HvGA20ox2* (Xu et al., 2017). All plants of the subset of lines showing height stability had the same *HvGA20ox1* allele, whereas both alleles were apparent for genotypes with high temperature response.

Focusing on the three extremely early genotypes we found an allelic variation in the *Ppd-H1* gene (**Paper I**). In addition to '247-11', '247-1', and 'Hrutur', the allele variation was also found in Scottish cv. 'Nairn', which was present in the pedigrees of all three early genotypes. *Ppd-H1* was not detected in the GWAS as the allele frequency was below the threshold for filtration (< 0.05). This illustrates one pitfall of relying on GWAS for finding loci, where the risk is that rare alleles (typically <5%) in the population will remain undetected. The classical method of QTL mapping in a segregating population will capture alleles from both parental lines, as their segregation naturally will provide a balanced dataset in the F1 generation. However, QTL-mapping is limited to capture only the diversity present among the two parental lines. A third approach, multi-parent advanced generation inter-cross (MAGIC) populations, have been increasingly employed in later years (Huang et al., 2015). In a MAGIC population several parental lines are inter-crossed to develop a population without structure (as in a classical segregating population) but with a multitude of diversity (as in a diversity panel). To elucidate better the genetic components, and to search for other rare alleles among Icelandic barley, a MAGIC population based on eight early parental lines has been developed and will be used to further advance our understanding of earliness at the northern margin of barley cultivation.

4.4. Study allelic diversity in selected loci by resequencing

We showed that two loci were of adaptive importance to induce earliness in Icelandic conditions (**Paper III**). The nomenclature of the alleles used below in the text (E1-3; P1-2; C1-2; F1-3) is explained in Figure 5. A winter type allele of *Ppd-H1* (allele P1) was found among the two earliest Icelandic genotypes '247-11' and '247-1'. These lines, however, were low yielding. The lines with the winter type allele were too few to fully conclude on the effect of *Ppd-H1* in the Icelandic genetic background, but our results indicate a trade-off with yield. Jones et al. (2008) showed a polymorphism in the *Ppd-H1* locus that causes non-responsiveness to long days (*ppd-H1*; (Haplotype P2)) in contrast to the wild-type allele *Ppd-H1* that is found in wild barley from the Fertile crescent. They further traced the *ppd-H1* allele to wild barley from Iran and Israel, and by studying haplotype diversity through re-sequencing of landraces they identified a proposed origin of the *ppd-H1* allele to a region in the Zagros Mountains, east of the Fertile crescent. This haplotype was later spread northwards in European landraces and has been found to follow a latitudinal gradient, where the non-responsive *ppd-H1* allele gives an adaptive advantage in northern latitudes compared to the wild-type *Ppd-H1* found in south Europe and in the Fertile crescent (Jones et al., 2008; Lister et al., 2009). We propose that at the northern margin of barley cultivation, the photoperiod responsive

allele, the wild-type *Ppd-H1* (P1), in a spring barley background, presumably in combination with *HvFT1* (allele F2, see below), can cause extreme earliness. This has been shown to have a yield penalty and is thus not found in commercial cultivars, but in breeding lines selected specifically for early heading in Iceland.

A winter type allele of *HvCEN* (C1) was found in four genotypes which were both early and high yielding. Again, the number of lines was low, but we propose that the allele is advantageous under Icelandic climatic conditions for a high and early yield. *HvCEN* has earlier been reported as having a strong latitudinal adaptation in a panel of barley from Europe, Asia, and Africa (Russell et al., 2016). Other studies have shown that it is virtually fixed in European two rowed spring barley (Tondelli et al., 2014). Fernández-Calleja et al. (2021) reviewed six major barley flowering genes and found that three *HvCEN* alleles (HI, HII, and HIII) were prevalent and shared between wild and domesticated barleys (with several more minor alleles). They found that allele 'HIII' was selected from wild barley and has since become fixed in European spring barley, whereas allele 'HII' is the dominant allele in wild barley from the Eastern Mediterranean and in cultivated winter barleys. *HvCEN* has, thus, been an important contributor to the expansion of barley into diverse habitats. We see a divergent pattern in the northern margin of possible barley cultivation, where the HII allele gives an adaptive advantage in a spring barley background, conferring improved earliness while maintaining the yielding capacity.

HvELF3 has earlier been proposed as of adaptive importance to the expansion of barley cultivation in Iceland (Zakhrabekova et al., 2012). We identified *HvELF3* as one of the significant MTAs in GWAS analysis (**Paper II**), which supports the claim from Zakhrabekova et al. 2012, however, in the re-sequencing (**Paper III**) we could not see any clear adaptive advantage from the allelic variants of *HvELF3*. It should be mentioned that we only explored variation in a region of exon 2, which is a limitation. Xia et al. (2017) has for example reported polymorphisms leading to amino acid changes in exon 4. Boden et al. (2014) reported increased GA production in *HvELF3* mutants, suggesting that *HvELF3* supresses heading under non-inductive photo periods by blocking GA production and *HvFT1* expression.

HvFT1 has not been proved to have an adaptive effect to northern latitudes and did not cause significant effects on earliness in our studies. Among four genotypes with the *ppd-H1* allele in a spring barley background, three were extremely early heading (see above), whereas the Scottish cv. 'Nairn' was not. In **Paper III**, we could see in the haplotype construction that 'Nairn' differed in its haplotype at the *HvFT1* locus. Hence, there could be an epistatic effect of *HvFT1* and *Ppd-H1*. It has earlier been reported that the long day responsive *Ppd-H1* allele upregulates *HvFT1* in long day conditions, whereas the long day non-responsive *ppd-H1* allele gives lower transcript levels of *HvFT1* (Fernandez-Calleja et al., 2021, and references therein). (Casas et al., 2011) constructed haplotypes in a set of Spanish landraces and found variation in the promotor and first intron. Loscos et al. (2014) found a copy number variation of *HvFT1* in the Finnish cv. 'Tammi' which corresponds to haplotype F2 in **Paper III**. This was the earliest allele in Loscos et al. (2014). We can see in Paper III that the genotypes with the *ppd-H1* allele differ in the *HvFT1* locus, where the extremely early Icelandic genotypes have the F2 allele, whereas the Scottish cv. 'Nairn' which is more average in earliness has the F1 allele. Thus, there seems to be a combined effect of P1 and F2 alleles that give the extreme earliness in Icelandic barley (Figure 5).

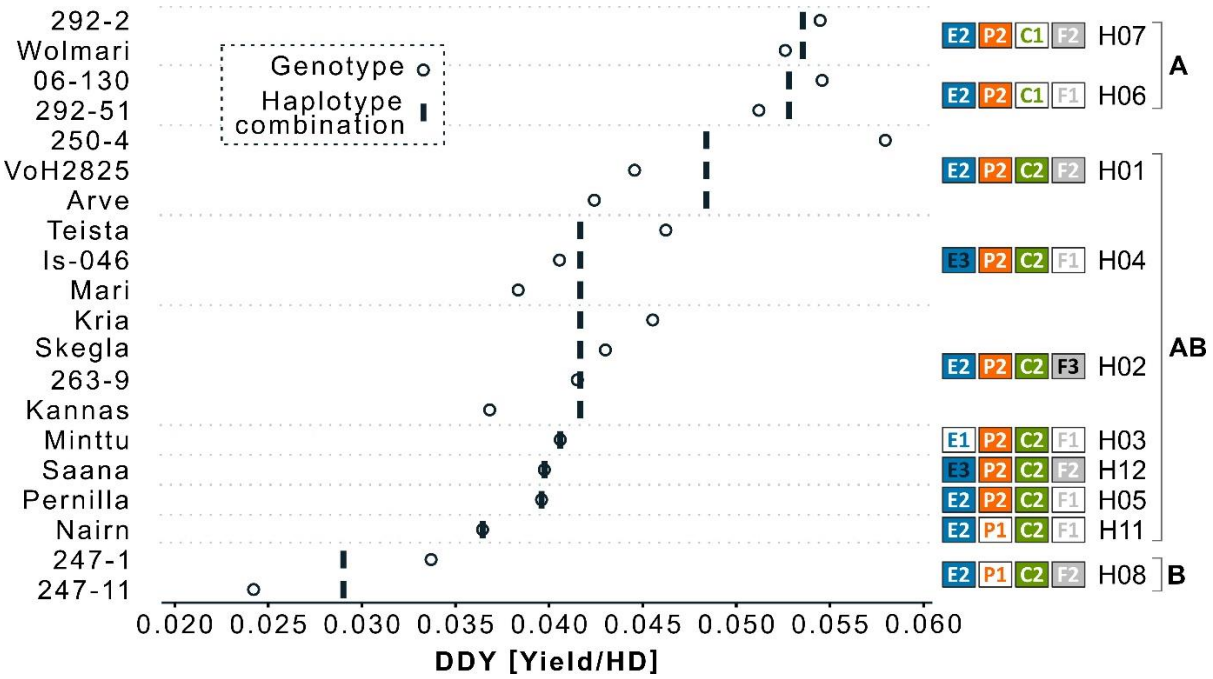

Figure 5. Index values of yield by heading day (DDY) for 20 barley genotypes. Open circles indicated DDY for each genotype. Vertical bands indicate the mean per haplotype combination. Haplotype combinations for the four flowering loci HvELF3 (blue, E), Ppd-H1 (orange, P), HvCEN (green, C), and HvFT1 (gray, F) are shown to the right of the diagram. Tukey's test for significance resulted in a significant difference between haplotype H08 and H07/H06 (p = 0.03) and is indicated with the letters to the far right.

4.5. Find allele combinations of adaptive importance for a northward expansion of barley cultivation

In **Paper I**, a first attempt to find favourable allele combinations was done, and in retrospective, more information would have been appropriate on the putative loci behind the marker trait associations. The allelic combination ACA was found significantly earlier maturing than other genotypes, and was a combination of the loci *HvGA20ox2*, and the putative loci *HvCry1b*, and *HvKAO1*. There is an inconsistency with regards to the localization of *HvCry1b* – and it may also be the *PhyA* locus, or the *Cytochrome P450*. Both *HvCry1b* and *HvPhyA* are involved in the photoreceptive pathway, where the plant circadian system registers the light wavelength. We have yet to elucidate the function of this allele.

HvKAO1 is a dwarfing gene involved in the GA pathway, as are *Cytochrome P450* and *HvGA20ox2*. KAO1 acts upstream of HvGA20ox2 in the GA pathway. The allelic combination thus looks like a combination of three alleles which all have a regulatory function in the GA synthesis pathway.

We found MTAs with *HvGA20ox2* (*denso/sdw1*) for height. This is not a novel finding but rather confirms the importance of the gene.

We can conclude that an allele combination made up of genes regulating the GA pathway are of importance in regulating both maturity and height in barley.

In **Paper II** an allele combination between *Ppd-H1*, *HvELF3*, and *HvFT1* with an average accumulated heat sum of 1,065°Cd for HSMD in the LDC environment (corresponding to 107 d) differed significantly ($p \leq .001$) from the overall mean of 1,251°Cd (corresponding to 125 d in LDC). That shows an effect of 186°Cd, corresponding to 18 days.

4.6. Trace the origin of adaptive alleles back in the pedigree

In the pedigree analysis of Paper III, a special emphasis was put on the extremely early sister lines '247-11' and '247-1'. The pedigree is reasonably well represented in the resequencing panel, and we can with a high level of confidence trace the P1 allele back to the Scottish cv. 'Nairn', which in turn got it from the Swedish winter barley cultivar 'Fimbul' which was released 1946 and was a cross between a Czechoslovakian winter barley and the Swedish landrace 'Gull'. 'Gull' was resequenced and did not carry the P1 allele, hence we can conclude that the P1 allele came from Czechoslovakia, via Sweden, then Scotland, to Iceland.

The emphasis on the early and high yielding lines ('292-2', '292-51', '06-130', and cv. 'Wolmari') began at a stage when the resequencing panel had already been selected, and hence, we lack crucial ancestral genotypes in the panel. We can conclude that the only other line carrying the C1 allele in the panel was the winter type cv. 'Fimbul', but 'Fimbul' does not occur in the pedigree of the three Icelandic lines ('292-2', '292-51', and '06-130'), and so is not the source of the C1 allele. One side of the pedigree comprises the Icelandic breeding line 'Is-046' with Swedish cultivars 'Arla' and 'Akka' (and further back 'Binder' and 'Gull', 'Tammi', and 'Maskin'), and Faroese landraces 'Tampar' and 'Sigur' in the pedigree, and they did not contribute the C1 allele. More resequencing of lines in the pedigree, for example the Norwegian cv. 'Olsok', could potentially shed light on this matter. 'Olsok', furthermore has the old cultivar 'Pirkka' from Finland, and the Swedish old cultivar 'Dore' selected from Swedish landraces from Jämtland in its pedigree.

5. Conclusion and future perspectives

Earlier studies have shown a latitudinal gradient in allelic diversity for *HvCEN* and *Ppd-H1* in European barley. The photoperiod insensitive allele *ppd-H1* enabled the northward expansion of barley in Europe by delaying the onset of flowering in spring sown barley genotypes. This gave more time in the vegetative stage and a better use of the whole growth season, and a higher yield. Likewise, allelic variation at *HvCEN* has a latitudinal effect where the winter type allele is beneficial in a Mediterranean environment. The early onset of flowering will make the barley escape the terminal summer drought. In contrast, the spring type allele is delaying the onset of flowering in a cool and humid environment in northern Europe, where it is beneficial for the barley plant to utilize the growing season, thereby maximizing the yield potential. In this project, it has been shown that in the extreme north, the pattern changes. In Iceland, winter type alleles of both *HvCEN* and *Ppd-H1* have a strong effect on earliness, and thereby the adaptation to produce mature seeds in the cold Icelandic summer. An allele combination with *HvFT1* and *Ppd-H1* produced extremely early, but low yielding genotypes. The winter type allele of *HvCEN* was found in genotypes which combined earliness with yield. The winter type allele of *HvCEN* could potentially be utilized in barley breeding programs to produce early maturing cultivars with a maintained yielding capacity. Further studies, preferably in a larger set of genotypes, would be beneficial to confirm the hypothesis.

HvGA20ox2 and *HvGA20ox1* were found to have effect on height. Their potential compensatory effects merits further studies, with special focus on height stability in contrasting temperature. Loci regulating the gibberellic acid pathway has an important role to play in both early maturity and plant height in spring barley bred for northern latitudes.

The novel insight of the genetics behind earliness provides a base to further adapt barley, and other cereals, to the climatic conditions of the extreme north.

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Paper I

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Identification of Ideal Allele Combinations for the Adaptation of Spring Barley to Northern Latitudes

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The northwards expansion of barley production requires adaptation to longer days, lower temperatures and stronger winds during the growing season. We have screened 169 lines of the current barley breeding gene pool in the Nordic region with regards to heading, maturity, height, and lodging under different environmental conditions in nineteen field trials over 3 years at eight locations in northern and central Europe. Through a genome-wide association scan we have linked phenotypic differences observed in multi-environment field trials (MET) to single nucleotide polymorphisms (SNP). We have identified an allele combination, only occurring among a few Icelandic lines, that affects heat sum to maturity and requires 214 growing degree days (GDD) less heat sum to maturity than the most common allele combination in the Nordic spring barley gene pool. This allele combination is beneficial in a cold environment, where autumn frost can destroy a late maturing harvest. Despite decades of intense breeding efforts relying heavily on the same germplasm, our results show that there still exists considerable variation within the current breeding gene pool and we identify ideal allele combinations for regional adaptation, which can facilitate the expansion of cereal cultivation even further northwards.

Keywords: earliness, GWAS, *Hordeum vulgare*, maturity, plant breeding, plant height, QTL

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INTRODUCTION

Climate change has begun to negatively affect yield of cereal crops (Lesk et al., 2016) and is predicted to cause even further yield losses in many of the low latitude grain producing regions of the world (Dai, 2011; Rosenzweig et al., 2014). At the same time global demand for cereals keeps growing (Tilman et al., 2011) caused by both population increase (Gerland et al., 2014) and altered consumption patterns (Kastner et al., 2012). The Nordic region is unique from an agricultural perspective with its relatively mild climate for its northern latitude and a long photoperiod during the growth season (Nurminiemi et al., 1996). Barley (*Hordeum vulgare* L.) is, alongside wheat, one

of the dominating cereal crops in the Nordic region[1], used primarily for feed and malt but with a growing demand for human consumption (Baik and Ullrich, 2008; Baik, 2016). Recent breeding efforts have paved the way for a more reliable barley harvest in the northern marginal area (Lillemo et al., 2010; Hilmarsson et al., 2017), where early flowering and the ability to reach maturity at low temperatures are key components to secure a high and stable yield at high latitudes (Nurminiemi et al., 1996; Hilmarsson et al., 2017). Events of strong winds and heavy precipitation are likely to increase in frequency due to global warming (Coumou and Rahmstorf, 2012). Hence, resistance to lodging and straw breaking are important traits. The heat wave in Scandinavia in the summer of 2018, which led to considerable yield losses, further stresses the importance to address a more volatile and unpredictable future climate. Here, early developing cultivars, which need less time in the field and are thus exposed to potentially damaging weather for a shorter time, could play a role in mitigating the risks. Better understanding of the genetics underlying these traits will enable breeders to produce locally adapted high yielding cultivars for the Nordic and sub-arctic region, further expanding the current cultivation area northwards.

Timing of flowering through seasonal cues, such as day length and temperature, is a key element for reproductive success (Andrés and Coupland, 2012). Earliness is a complex trait where genetic variation can greatly alter the plants response to photoperiod and temperature (Cockram et al., 2007; Blümel et al., 2015). In its region of origin, barley germinates in the fall and stays in the vegetative phase during the cool and humid winter season; increased day length in the spring triggers the onset of flowering and the plants mature at the start of the dry summer period ensuring a period of dormancy for the seeds during the hot and dry summer (Lister et al., 2009). Consistent with its importance for the plant's survival the response to changes in the photoperiod is controlled by several well conserved genes (Blümel et al., 2015). Among those is the *Ppd-H1* gene, located on chromosome 2H, whose wild type function is to promote flowering under long day conditions (Turner et al., 2005; Jones et al., 2008; Loscos et al., 2014). With the expansion of barley northward with the spread of agriculture, a recessive *ppd-H1* allele with delayed flowering was favored (Jones et al., 2008). This recessive allele helped the spring barley utilize the summer season in the northern latitudes by a less strong up-regulation of the *HvFT1* gene than with the wild type *Ppd-H1* allele (Hemming et al., 2008). Lister et al. (2009) found a latitudinal increase in the prevalence of this recessive allele in historical cultivars and landraces from Europe. Another important gene for the regulation of flowering is *HvCO1*, a gene acting in parallel with *Ppd-H1*, whose overexpression leads to up-regulation of the *HvFT1* gene, which in turn leads to flowering (Campoli et al., 2012; Loscos et al., 2014). *HvCO2* on chromosome 6H is a paralog to *HvCO1* (Campoli et al., 2012). *HvFT1* has alleles with copy number variation (CNV) which have been associated with early flowering in spring barley, a phenotype first discovered in the Finnish cultivar Tammi (Nitcher et al., 2013; Loscos et al., 2014). *HvFT1* has two paralogs: *Ppd-H2* (synonym *HvFT3*) which promotes spikelet initiation (Mulki et al., 2018) and *HvCEN*, which has been shown to affect flowering time and has a latitudinal specific distribution of alleles suggesting adaptive function in the northwards range expansion of barley (Comadran et al., 2012). The *HvCEN* (syn. *eps2S* or *eam6*; Comadran et al., 2012; Alqudah et al., 2016) locus inhibits flowering and is located in the centromeric region of chromosome 2H (Comadran et al., 2012). *HvCEN* has been shown to have a mutant allele that, contrary to the wild type allele, does not inhibit flowering in spring barley and interact with *HvFT1* (Loscos et al., 2014). Another *FT*-like gene is HvFT4 on the short arm of chromosome 2H which is a temperature responsive gene with increased expression in high temperature (Ford et al., 2016). *HvELF3* (syn. *Mat-a* or *Eam8*) on chromosome 1H is a homolog of *Arabidopsis thaliana* gene *ELF3* (Zakhrabekova et al., 2012). The dominant *HvELF3* allele delays flowering in long day conditions while several of the recessive alleles provide day length neutrality which leads to early flowering in both long-day and short-day conditions (Faure et al., 2012). One recessive allele (*mat-a.8*) is the result of an induced mutation in the cultivar Bonus and was released 1960 with the cultivar Mari (Gustafsson et al., 1971; Lundqvist, 2009), the name describing its main characteristics (from Latin for *ma*tura = early and *ri*gida = stiff) (Gustafsson et al., 1971). The day length neutrality associated with the *HvELF3* polymorphism has been proposed to enable cultivation of barley as far north as Iceland, as well as enabling the spread of barley to high altitude regions near the equator (Faure et al., 2012; Zakhrabekova et al., 2012). *Vrn-H1* (*HvAP1*) on chromosome 5H is involved in vernalization requirement and interacts with *Vrn-H2* on chromosome 4H. A wild type recessive *vrn-H1* and a functional *Vrn-H2* always result in a winter growth habit (Karsai et al., 2005; Loscos et al., 2014). Several alleles of *Vrn-H1* exists, resulting in a spring growth habit or a facultative growth habit in lines with a spring allele in *Vrn-H1* or lines where *Vrn-H2* is deleted (Loscos et al., 2014).

In addition to the importance of the correct timing of flowering, plant height matters as demonstrated when dwarfing genes were introduced into wheat in the green revolution (Peng et al., 1999). Intensive cereal cultivation is today dependent on semi-dwarf cultivars (Kuczyńska et al., 2013) since short and strong stems help the plants withstand wind, prevent lodging and can positively affect the harvest index (Hay, 1995). Studies have shown that height reduction is the result of either reduced hormone expression or hormone insensitivity (Dockter et al., 2014), and that two of the main factors regulating plant height are the plant hormones brassinosteroids (BRs) and gibberellic acids (GAs) (Marzec and Alqudah, 2018). BRs are also known to affect traits such as tiller number and grain size in rice (Zhang et al., 2014). In barley, height is controlled by dwarfing and semi-dwarfing genes as well as other genes affecting plant height (Wang et al., 2014). The dwarfing genes are not useful in breeding as they are linked to reduced vigor and yield (Wang et al., 2014). Instead, semi-dwarfing genes have been widely employed in modern barley breeding (Kuczyńska et al., 2013; Wang et al., 2014), these include semi-brachytic 1 (*uzu1*) (Chono et al., 2003), *semi-dwarf 1* (*sdw1*/*denso*) (Jia et al., 2009), *breviaristatum-e* (*ari-e*) (Liu et al., 2014), and short culm 1 (*hcm1*) (Wang et al., 2014). The *uzu1* and *sdw1*/*denso* genes

[1]http://faostat3.fao.org/

are both located close to the centromere on chromosomal arm 3HL with the *sdw1/denso* gene located more distally from the centromere. The *ari-e* locus is located on chromosomal arm 5HL and the *hcm1* gene is located on chromosomal arm 2HL (Wang et al., 2014). Modern European barley cultivars generally depend on the *sdw1/denso* locus as their source of semi-dwarfing (Kuczyńska et al., 2013). Plants carrying the semi-dwarf allele of the *sdw1/denso* locus can be identified morphologically by having a prostrate growth habit in their juvenile stage, whereas plants carrying the dominant allele have an erect juvenile growth habit (Kuczyńska et al., 2013).

High-throughput genotyping has developed as a feasible alternative to traditional genotyping with molecular markers, such as AFLPs. The high-throughput method utilizes single nucleotide polymorphisms (SNP) spread across the genome at an even distribution (Comadran et al., 2012). The development of high-throughput SNP-panels enables a genomic resolution not easily obtained by other marker types. This improved coverage has opened up the possibility to perform genome wide association scans (GWAS) on a variety of agricultural traits (see e.g., Waugh et al., 2014).

Linkage disequilibrium (LD) is the non-random co-segregation of alleles at two loci (Flint-Garcia et al., 2003). LD is generally higher in self-pollinating crops than in out-breeding species and is higher in homogenous than in diverse populations (Flint-Garcia et al., 2003). In association mapping, LD affects the number of markers needed as well as the resolution obtained in the associations (Rafalski, 2002). In self-pollinating crops with very high LD the resolution gets lower as associated markers may be located far away from the responsible locus (Malysheva-Otto et al., 2006). In contrast, when LD is low, the resolution is high as the distance between associated marker and the gene of interest will be short (Remington et al., 2001). A recent study of the same Nordic population as used here confirmed the average LD to be in the range 0–4 cM but with large variations in different chromosomal regions and varying among the population structure groups (Bengtsson et al., 2017).

Better understanding of the allelic diversity in the Nordic breeding material will enable the application of marker-assisted selection for beneficial allele combinations and speed up the breeding process. Detailed understanding of loci controlling earliness and straw stability will enable fine-tuning of cultivars better adapted to northern latitudes. We screened a panel of 169 barley lines from the Nordic breeding pool at eight locations for the traits heading day, maturity day and straw stability with the aim of performing a genome-wide association analysis to identify loci responsible for the quantitative traits earliness and straw stability in multiple environments.

MATERIALS AND METHODS

Plant Material, Test Locations, and Phenotyping

A panel of 169 spring barley lines, representing the Nordic breeding gene pool, were included in the study, selected by each

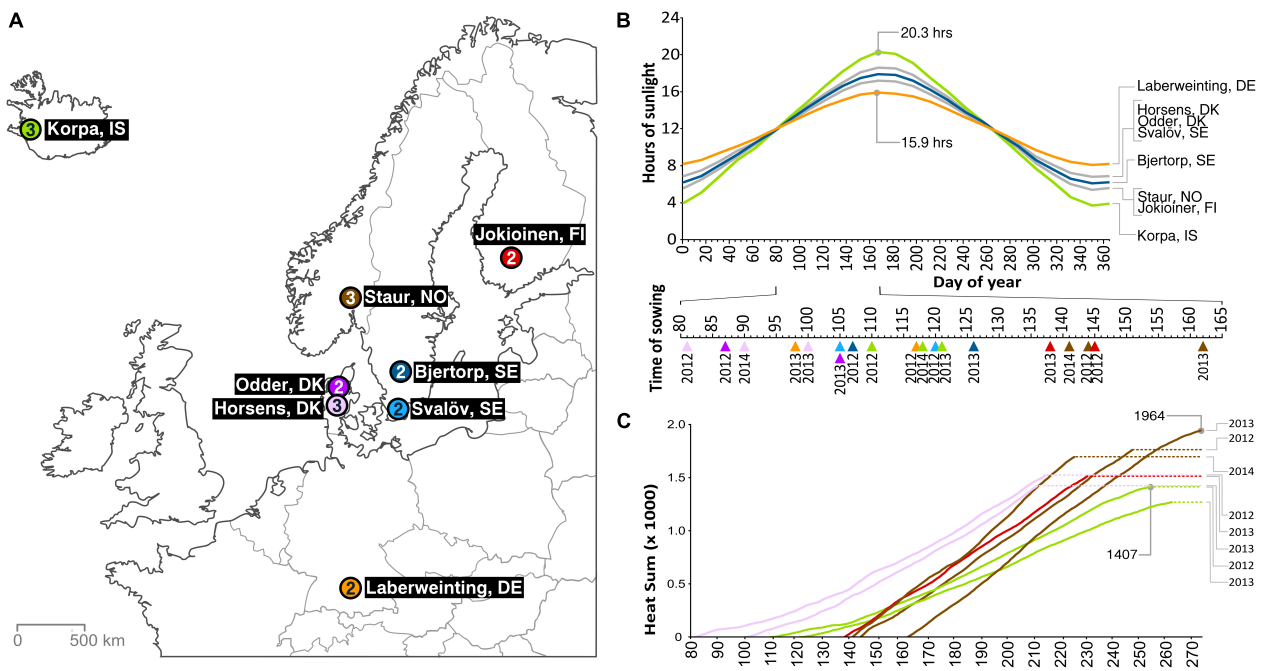

FIGURE 1 | An overview of the multi-environment field trials (MET). **(A)** Field tests were performed at eight different locations in six different countries, ranging from Laberweinting, Germany in the south to Korpa, Iceland in the north, for up to 3 years, resulting in 19 distinct environments. The number of years is shown inside circles. **(B)** The different day lengths between test locations, with the respective sowing days of the trials. **(C)** The accumulated heat sum (°C) from sowing day until maturity of the latest maturing line in the eight trials where maturity was screened. The highest and lowest value are shown in the graph (at Korpa, IS 2013 not all lines reached maturity).

of six different Nordic barley breeding entities (**Supplementary Table S1**). The main aim of the selection process was to maximize diversity for pathogen resistance, earliness and straw quality, among advanced cultivars and breeding lines. Out of the 169 lines – 124 two-rowed and 45 six-rowed – 58 lines were of Danish origin (all two-rowed), 30 Swedish (28 two-rowed and 2 six-rowed), 30 Norwegian (3 two-rowed and 27 six-rowed), 29 Finnish (all two-rowed), 21 Icelandic (5 two-rowed and 16 six-rowed), and one from the United Kingdom (two-rowed). The two panels used in this study, one consisting of all 169 lines and one with only the 124 two-rowed lines, are referred to as PPP169 and PPP124, respectively.

Multi-environment field trials (MET) were performed in eight locations for 2–3 years, resulting in a maximum of 19 distinct environments (**Figure 1A** and **Supplementary Table S1**), ranging from Laberweinting, Germany in the south (48°48′6″N) to Korpa, Iceland in the north (64°08′56″N) and Jokioinen, Finland in the east (23°29′54″E) to Korpa in the west (21°45′03″W). Within the environments there is great variation in hours of sunlight during the growth period (**Figure 1B**) and the heat sum available (**Figure 1C**). The field trials were set up with up to three replications in an alpha lattice design. Plot size varied between locations from row sowings up to 10 m^2 field plots. Lodging and straw breaking were not recorded in row sowings, since it could have yielded a different result compared with field plots.

Plants were phenotyped for early spring growth in four environments (measured as height in all lines when the first lines entered growth stage 31, 32, and 34: Ht31, Ht32, and Ht34, respectively) (Zadoks et al., 1974). The measurement was conducted from soil level up to a cardboard plate laid on top of the plants in the plot, as an estimator of the height. Heading day (HD) was recorded in 17 environments as number of days from sowing until half of the spike was visible in 50% of the plants in each plot (stage 53; Zadoks et al., 1974). Maturity day (MD) was recorded in 7 environments as number of days from sowing until the peduncle below the spike turned yellow (approximate growth stage 89; Zadoks et al., 1974). Grain filling period (GFP) was calculated as the period from HD to MD (7 environments). Heat sums for HD (HSHD), MD (HSMD), and GFP (HSGFP) were calculated by adding the maximum daily temperature with the minimum daily temperature and dividing them by 2, then adding the sums for each day of the respective period giving the growing degree-days (GDD), with 0°C used as base line temperature, meaning only sums above 0°C were added. Straw length (StL) was measured upon maturity from soil level to below the spike in cm (11 environments). Lodging (Ld), recorded in 6 environments and straw breaking (SB), recorded in 5 environments were recorded on a scale from 1 to 9 where 1 means no lodging/straw breaking, 5 means 50% lodging/straw breaking and 9 means 100% lodging/straw breaking.

DNA Extraction and Genotyping

DNA was extracted using a standard CTAB protocol (Cetyl Trimethyl Ammonium Bromide) with DNA extraction kits as described earlier by Orabi et al. (2014). All lines were genotyped with the barley 9K iSelect SNP chip which contains 7842 SNP markers (Comadran et al., 2012). Genotyping was performed by TraitGenetics (Gatersleben, Germany).

6208 SNP markers were polymorphic >5% level and their physical position (in base pairs) on the barley reference genome (Mascher et al., 2017) were retrieved using the online tool BARLEYMAP[2] (Cantalapiedra et al., 2015).

Descriptive Statistics

All traits were continuous except for the discrete character row type. For the continuous characters, a normal distribution was expected. To check for deviations from the expected normal distribution, data for all trials and traits were plotted in distribution plots using Excel Add-In XLSTAT v. 19.2. Pearson correlations were calculated to describe the relationships between traits and between field trials using Microsoft Excel 2016.

Descriptive statistics for all environments, separately and combined, were computed with the psych software package v. 1.8.12 (Revelle, 2018) using the R software (R Development Core Team, 2017), this included number of observations (n), mean, standard deviation (sd), median, median absolute deviation (mad), minimum (min), maximum (max), range, skew, kurtosis, and standard error (se).

Analysis of Variance (ANOVA)

To evaluate the relative contributions of genotype, environment, and genotype by environment interactions in the data set, each trait was analyzed with mixed linear modeling using PROC MIXED in SAS v. 9.4 (SAS Institute Inc.). This initial analysis assumed genotypes, environments, and the genotype by environment interactions to be fixed effects and replications and blocks within replications of the individual trials as random effects.

Best linear unbiased estimates (BLUEs) to be used in the GWAS analyses were calculated using the lmer function in the "lme4" R package (Bates et al., 2015), assuming all effects, except the genotypic effects to be random. Phenotypic data across years were estimated as:

$$y_{ijk} = \mu + G_i + en_j + r_{(j)k} + e_{ijk},$$

where y_{ijk} is the kth observation of the ith genotype in the jth environment, μ is the common intercept, G_i is the effect of the ith genotype, en_j is the effect of the jth environment, $r_{(j)k}$ is the effect of the kth replication in environment j, and e_{ijk} is the corresponding error.

Population Structure

Population structure was evaluated with the software STRUCTURE v.2.3.4 (Falush et al., 2007) and by principal component analysis (PCA) using GenAlEx v.6.5.0.1 (Peakall and Smouse, 2006; Peakall and Smouse, 2012; as in Bengtsson et al., 2017).

For the STRUCTURE analysis, the SNP genotype data was run 10 times with a burn-in period of 9999 followed by 9999 iterations from K = 1 to K = 12. To identify the optimal number of genetic clusters (subpopulations), ΔK values were calculated as proposed

[2]http://floresta.eead.csic.es/barleymap/

by Evanno et al. (2005) using STRUCTURE HARVESTER v. 0.6.94 (accessed 25 Nov. 2015)[3]. The STRUCTURE analysis was previously run for the PPP169 panel in Bengtsson et al. (2017), but here an identical analysis for the PPP124 panel was run. An analysis of molecular variance (AMOVA) was run in GenAlEx to check for variance among the STRUCTURE groups.

Genome-Wide Association Analysis

Genome-wide association analysis was performed using TASSEL v. 5.2.31 (Bradbury et al., 2007) and using the R package, Genome Association and Prediction Integrated Tool (GAPIT v. 3.0) (Lipka et al., 2012). The hapmap file of the SNP markers was filtered to exclude unsuccessful marker assays, monomorphic markers, and rare alleles (with less than 5% occurrence in the population). Unmapped SNP markers were assigned to an artificial chromosome to capture any associations with these markers.

After filtering 5710 SNP markers remained in the analysis of the PPP169 panel, or 73% of the total number of 7864 SNPs. When the PPP124 panel was filtered to remove markers below 5% polymorphism, 5037 SNP markers remained. Kinship matrices were constructed based on the filtered set of markers using the scaled identity-by-state (IBS) method (Zhang et al., 2010) for both panels. The respective kinship matrices were then used in subsequent mixed linear model (MLM) analyses (Yu et al., 2006; Zhang et al., 2010). To select the correct model and account for population structure we performed four associations using TASSEL: (1) General linear model (GLM) analysis without including population structure in the model; (2) Efficient mixed model association (EMMA) using the kinship matrix with *Q*-values from STRUCTURE K = 2; (3) EMMA using kinship matrix with eigenvalues from the PCA analysis; and (4) EMMA using only the kinship matrix. In addition, the models in TASSEL were compared with the following models using GAPIT v. 3.0: (5) MLM using the van Raden kinship; and (6) MLM using the van Raden kinship with eigenvalues from the PCA analysis. The significant allelic effect estimate is given in relation to the minor allele in the GAPIT output. The MLM was run as optimum level and the P3D (estimated once) variance component estimation. Quantile-quantile (Q-Q) plots were created by comparing expected and observed chi-square values and Manhattan plots showing positions of associated markers across the genome were constructed for each trait using the R package CMplot[4]. Quantile-quantile plots (Q-Q plots) were plotted to assess the goodness of fit of the model for each trait. Large deviations from the expected distribution mean that the model does not fit the data. The models were evaluated trait-wise by comparing the Q-Q plots and checking the narrow sense heritability values, where a higher heritability indicated that the model had a higher predictive value.

GWAS was performed trait-wise on the calculated BLUEs from all trials with the PPP169 panel, as well as on the PPP124 panel which includes only the two-rowed lines. A threshold value was calculated to estimate a significance level for the association analysis. The Bonferroni method based on the total number of markers for each panel and a significance level of 0.05 gave a $-\log_{10}(p) = 5.00$ for the PPP124 panel and $-\log_{10}(p) = 5.06$ for the PPP169 panel. This is a very stringent method (Gupta et al., 2014) considering that many of the markers are strongly linked. Thus, a suggestive threshold, earlier published by Duggal et al. (2008), allowing for one false positive per genome scan was estimated by dividing 1 by the number of markers for each panel. This resulted in a suggestive threshold of $-\log_{10}(p) = 3.70$ for PPP124 and $-\log_{10}(p) = 3.76$ for PPP169, which is considered as the significance threshold for the marker-trait associations identified in this study. Both thresholds are indicated in the Manhattan plots.

The length of the quantitative trait loci (QTL) were decided by calculating the LD between the most significant markers at each intra-chromosomal locus using the TASSEL software. The r^2-values earlier reported for each chromosome and each panel (Bengtsson et al., 2017, **Supplementary Tables S3**) were used as threshold values for determining whether a QTL should be regarded as distinct or not.

Allele Frequencies and Combinations

Allele combinations were constructed for the traits Ht34, HSHD, HSMD, StL, and SB in the PPP169 panel. The most significantly marker-trait associated SNP marker was used for each QTL and for practical reasons, the total combination of SNPs used to construct the allele combinations was limited to three. The effect of each allele combination with at least five observations (lines) was calculated based on BLUE values and the significance of the effects was tested using the lm () function in R. Allele frequencies were calculated for the SNP markers used in construction of the allele combinations, as well as previously described SNP markers for earliness traits (Comadran et al., 2012; Maurer et al., 2015).

RESULTS

Descriptive Statistics and Distributions

Summary statistics were calculated for all nineteen environments, individually and combined (**Supplementary Tables S2**, **S3**), and frequency distributions plotted for all phenotypic traits (**Supplementary Figure S1**). For early vigor (Ht31, Ht32, Ht34), the distributions were generally right-skewed indicating mostly late genotypes in the two panels, with a few very early developing lines. StL, SB, and Ld had normal distributions in both panels. For earliness traits (HD, HSHD, MD, HSMD, GFP, and HSGFP) the distributions were left-skewed, indicating a few early lines with the majority being later developing in both panels. The earliest genotypes were all six rowed, hence were not represented in the PPP124 panel.

Analysis of Variance and Correlations

For all traits in both panels the genotype, environment, and genotype by environment interaction were significant ($p < 0.05$) except for environment effect in Ht32 in the PPP124 panel (**Supplementary Table S4**). The results showed that the effect of genotype by far outweighed the genotype by environment interaction for all traits. Pearson correlations performed on overall means for all 12 traits (**Table 1**) showed

[3]http://taylor0.biology.ucla.edu/structureHarvester/

[4]https://github.com/YinLiLin/R-Cmplot

that the earliness traits (HD, HSHD, MD, HSMD, GFP, and HSGFP) were all positively correlated ($p < 0.01$), but with negative correlations to the straw properties and the early vigor (StL, SB, Ld, Ht31, Ht32, and Ht34), these were in turn positively intercorrelated.

Pearson correlations performed trait-wise between trials (**Supplementary Table S5**) showed significant correlation between trials for earliness traits and early vigor. Subsequently, these traits were analyzed as means of all trials in the GWAS analyses. More variation was found for straw properties, where single trials were not correlated with the rest. In these cases, trials which had significant correlation were analyzed as means, whereas trials without significant correlation were analyzed separately.

Population Structure

The STRUCTURE analysis for the PPP169 panel analyzed in Bengtsson et al. (2017) gave a maximum ΔK value at K = 2, where K1 comprised two-rowed lines from southern regions (mainly Finland, southern Sweden, and Denmark), K2 comprised the six-rowed lines and the admixed group was comprised of two-rowed lines from Norway, Iceland and northern Sweden. Here, STRUCTURE analysis of the PPP124 panel revealed the most variation for two subpopulations and AMOVA showed 38% of the genetic variation explained between K groups. If the two STRUCTURE groups were further subdivided according to Tondelli et al. (2013) with an admixed group with less than 0.7 proportion of the genetic variation assigned to K1 or K2, respectively, these three groups explain 35% of the total variation. For practical purposes, the admixed genotypes were assigned to groups, either north-western or south-eastern, based on knowledge of breeding entity.

Genome-Wide Association Analysis

GWAS was run for 13 and 12 traits for the PPP169 and PPP124 panels, respectively. The best model, of the six models evaluated, was selected for each trait following the criteria mentioned in material and methods (**Supplementary Table S6**). For all but two traits MLM with the van Raden kinship matrix, was used. For Ld in both panels, MLM with van Raden kinship matrix and eigenvalues from the PCA were used to account for population structure. Results from analyses using MLM van Raden in GAPIT were reported, due to the resulting associations, where GAPIT in a few cases (e.g., for spike type) yielded more peaks that passed the significance threshold and could be explained by known loci for the respective traits.

In total, for all 12 traits analyzed (excluding spike morphology), 108 significant markers with known genetic

TABLE 1 | Pearson pairwise correlations of overall means for days from sowing to heading (HD), accumulated heat sum from sowing to heading (HSHD), days from sowing to maturity (MD), accumulated heat sum from sowing to maturity (HSMD), grain filling period (registered as the number of days between heading and maturity) (GFP), and the accumulated heat sum in the grain filling period (HSGFP), early vigor (measured as height at growth stage 31, 32, and 34 (Zadoks et al., 1974) (Ht31, Ht32, and Ht34), straw length (StL), straw breaking (SB), and lodging (Ld) for both panel PPP124 and PPP169.

	HD	HSHD	MD	HSMD	GFP	HSGFP	Ht31	Ht32	Ht34	StL	SB
PPP124											
HSHD	0.990										
MD	0.865	0.861									
HSMD	0.871	0.867	0.997								
GFP	0.552	0.524	0.870	0.860							
HSGFP	0.449	0.413	0.769	0.770	0.906						
Ht31	−0.694	−0.698	−0.802	−0.798	−0.676	−0.598					
Ht32	−0.705	−0.704	−0.803	−0.800	−0.665	−0.598	0.957				
Ht34	−0.742	−0.741	−0.828	−0.823	−0.672	−0.595	0.948	0.968			
StL	−0.393	−0.416	−0.459	−0.450	−0.357	−0.251	0.537	0.585	0.639		
SB	−0.458	−0.455	−0.558	−0.558	−0.492	−0.435	0.469	0.462	0.503	0.289	
Ld	−0.376	−0.359	−0.433	−0.437	−0.368	−0.338	0.417	0.460	0.509	0.532	0.550
PPP169											
HSHD	0.995										
MD	0.930	0.932									
HSMD	0.930	0.932	0.998								
GFP	0.496	0.510	0.780	0.776							
HSGFP	0.587	0.584	0.833	0.839	0.971						
Ht31	−0.811	−0.802	−0.804	−0.807	−0.519	−0.601					
Ht32	−0.835	−0.826	−0.829	−0.830	−0.538	−0.618	0.980				
Ht34	−0.869	−0.862	−0.869	−0.868	−0.575	−0.648	0.962	0.981			
StL	−0.379	−0.394	−0.409	−0.406	−0.321	−0.317	0.534	0.570	0.607		
SB	−0.730	−0.736	−0.747	−0.748	−0.523	−0.570	0.707	0.714	0.753	0.542	
Ld	−0.329	−0.324	−0.382	−0.383	−0.344	−0.372	0.342	0.388	0.427	0.566	0.467

All correlations were significant ($p \leq 0.01$).

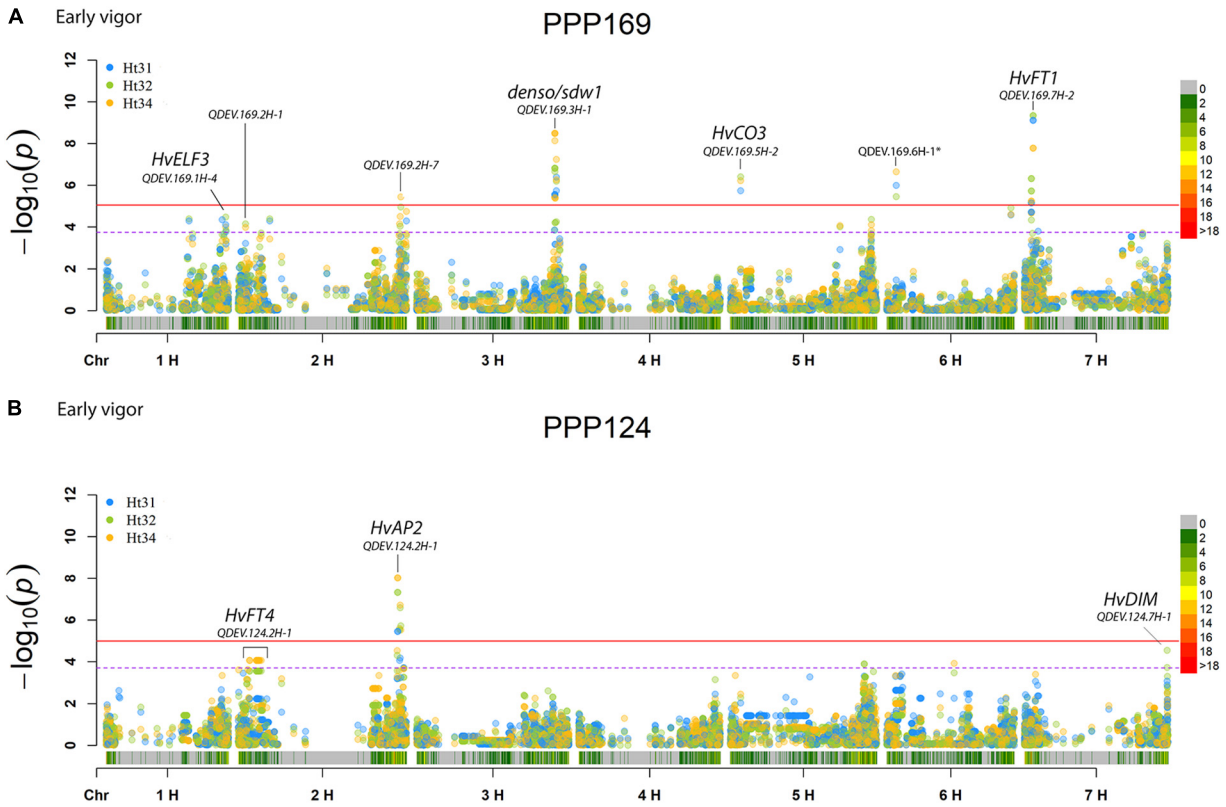

FIGURE 2 | GWAS results for the traits early spring growth stages 31, 32, and 34 (Zadoks et al., 1974), for panels **(A)** PPP169 and **(B)** PPP124, respectively. The Manhattan plots show significant associations (Bonferroni threshold in red, and the suggestive threshold in purple) between trait and marker, the x-axis shows the physical distance over all seven barley chromosomes. The bar under the x-axis shows the SNP distribution on each chromosome, where 0 −>18 depicts SNP density (the number of SNPs per 1 Mbp bin). The non-significant and significant associations are displayed as open and solid filled circles, respectively. The significance of the associations at values between these two extremes are displayed as circles with varying amounts of fill. Relevant QTL names and putative loci are presented in the figure. Note that the * indicates a SNP marker located at 3H position 105 cM using the POPSEQ 2017 reference map (http://floresta.eead.csic.es/barleymap/).

position were found with 50 and 45 markers unique for the PPP169 and PPP124 panels, respectively (**Supplementary Tables S7**, **S8**). In total 23 and 11 QTL were found in the PPP169 and PPP124 panels, respectively. GWAS results are presented for nine out of twelve traits analyzed, that is for early spring growth stages 31, 32, and 34 (**Figure 2**), StL, straw breaking and lodging (**Figure 3**), and heat sum heading, heat sum maturity, and heat sum GFP (**Figure 4**). Manhattan plots of spike morphology are shown for the PPP169 panel (**Figure 5**).

Allelic Diversity and Allele Combinations

Observed allelic diversity for selected SNP markers from the GWAS analyses, and for a set of SNP markers that have previously been associated with flowering genes (Comadran et al., 2012; Maurer et al., 2015), showed different patterns of polymorphisms between the two geographic groups. Several loci were effectively fixed in the south-eastern lines, for example markers nearby or in the *Ppd-H1, HvCO1, HvCO3, HvFT1*, and *denso/sdw1* loci (**Supplementary Figure S2**). In contrast, alleles for markers nearby the *Vrn-H1* locus were fixed in the north-western lines (**Supplementary Figure S2**).

Allele combinations, with significance levels for the effects, were constructed for the traits Ht34, HSHD, HSMD, StL, and SB in the PPP169 panel (**Figure 6** and **Supplementary Figure S3**).

DISCUSSION

Latitudinal Adaptation of Earliness in Nordic Spring Barley

The barley lines analyzed here could be split in two based on origin, that is into a north-western group and a south-eastern group. The south-eastern group comprised 110 genotypes from breeders in Denmark, Finland, and southern Sweden. The north-western group comprised 58 genotypes from breeders in northern Sweden, Norway, and Iceland. The single genotype from the United Kingdom was not included in the geographical grouping. Despite the north-western group including fewer lines, more diversity was observed there, possibly due to the fact that six-rowed lines were almost exclusively found in this group. When looking only at the PPP124 panel (all two-rows), the pattern was less clear.

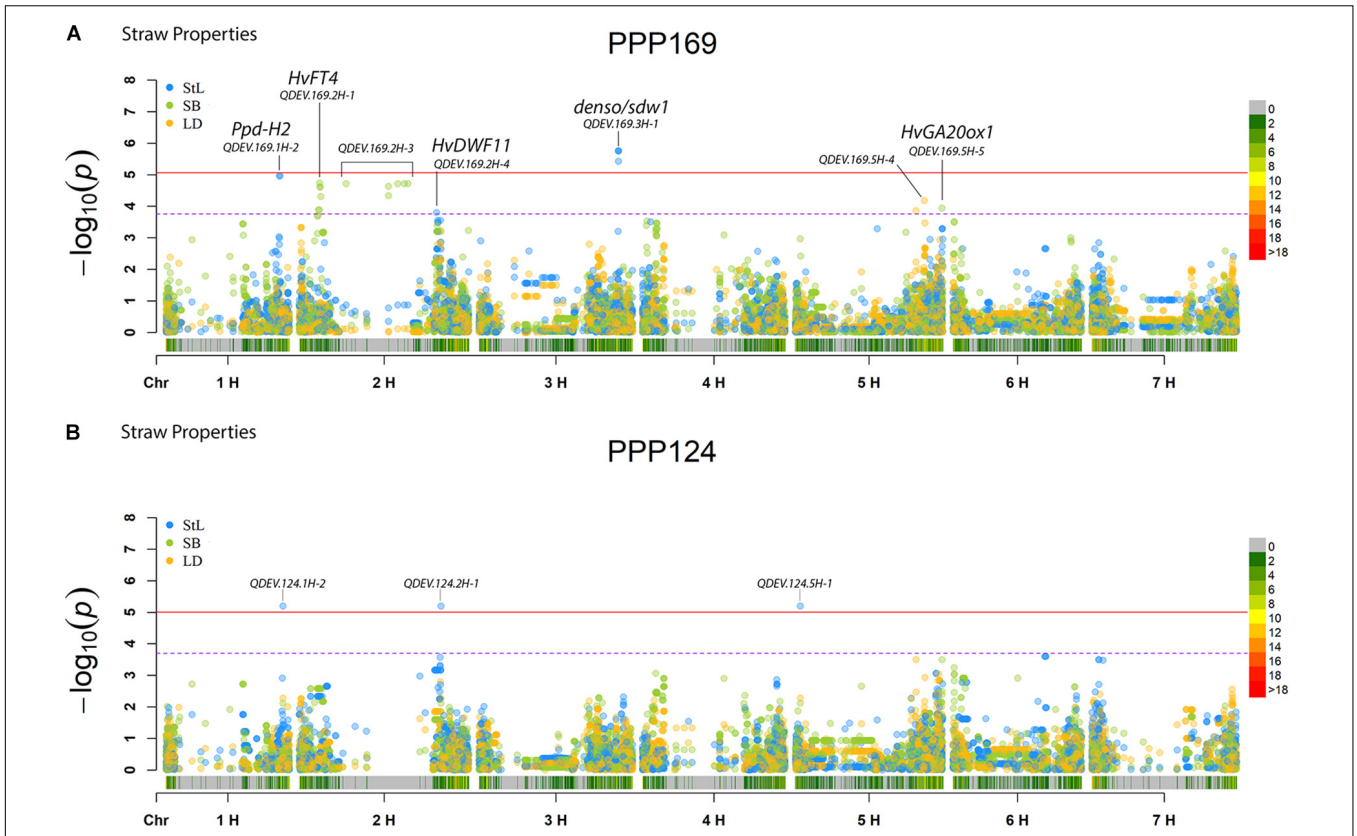

FIGURE 3 | GWAS results for the traits straw length, straw breaking and lodging for panels **(A)** PPP169 and **(B)** PPP124, respectively. The Manhattan plots show significant associations (Bonferroni threshold in red, and the suggestive threshold in purple) between trait and marker, the x-axis shows the physical distance over all seven barley chromosomes. The bar under the x-axis shows the SNP distribution on each chromosome, where 0 -> 18 depicts SNP density (the number of SNPs per 1 Mbp bin). The non-significant and significant associations are displayed as open and solid filled circles, respectively. The significance of the associations at values between these two extremes are displayed as circles with varying amounts of fill. The most significant values, located at the top, are displayed as solid filled dots. The significance of the associations at values between these two extremes is indicated by all other dots with varying amounts of fill. Relevant QTL names and putative loci are presented in the figure.

A few of the well-known earliness loci had fixed alleles in the two-rowed lines, for example markers in or nearby the *Ppd-H1* and *HvCEN* locus, which could explain why these loci were not detected in the GWAS (**Supplementary Figure S2**). This is in accordance with previous findings that the wild type *Ppd-H1* and *HvCEN* alleles are fixed in European two-rowed spring barley (Tondelli et al., 2013). *HvCEN* has also been reported as the locus most strongly associated with latitudinal effect among 19 tested flowering associated loci in a European landrace collection (Russell et al., 2016).

Three chromosomal regions have earlier been shown to be of importance in the regulation of flowering in barley; namely chromosomal arm 1HL (*HvELF3* and *Ppd-H2)*, the short and long arm of chromosome 2H (*Ppd-H1* and *HvCEN),* and chromosomal arm 7HS (*HvFT1* and *HvCO1)* (Loscos et al., 2014). Here we report associations on 1HL for early vigor in the PPP169 panel, but not in the PPP124 panel, with QDEV.169.1H-4 located only 4 Mbp (2.6 cM) from the *HvELF3* locus (**Figure 2A**). QDEV.169.1H-4 showed diversity in the south-eastern two-rowed lines but had fixed alleles in the north-western two-rowed lines indicating that this locus might have an associated adaptive advantage with increasing latitude.

The strongest association for early vigor in the PPP169 panel (QDEV.169.7H-2) was found on chromosomal arm 7HS. The two most significant markers (both showing an identical pattern) at the 7H-2 QTL, 12_30894, and 12_30895, were both located within the *HvFT1* locus (**Supplementary Table S7**). The pattern of allelic diversity reflected the geographical origin where both alleles were present among the north-western lines, whereas in the south-eastern lines the allele was fixed (**Supplementary Figure S2**). Although, the *HvCO1* gene could not be detected in the GWAS analysis, the allele frequency for the known *HvCO1* marker, BK_03 (Comadran et al., 2012), showed a similar pattern as that seen for QDEV.169.7H-2.

Another QTL with strong association (QDEV.169.3H-1) to early vigor, heading, and maturity was detected on chromosomal arm 3HL nearby the known flowering and semi-dwarf locus, *denso/sdw1*, in the PPP169 panel (**Figures 2**, **4**). The QDEV.169.3H-1 had a too low allele frequency in the PPP124 panel (<0.05 MAF) to be detected in the GWAS. The *denso/sdw1* locus has been speculated to have an effect on both

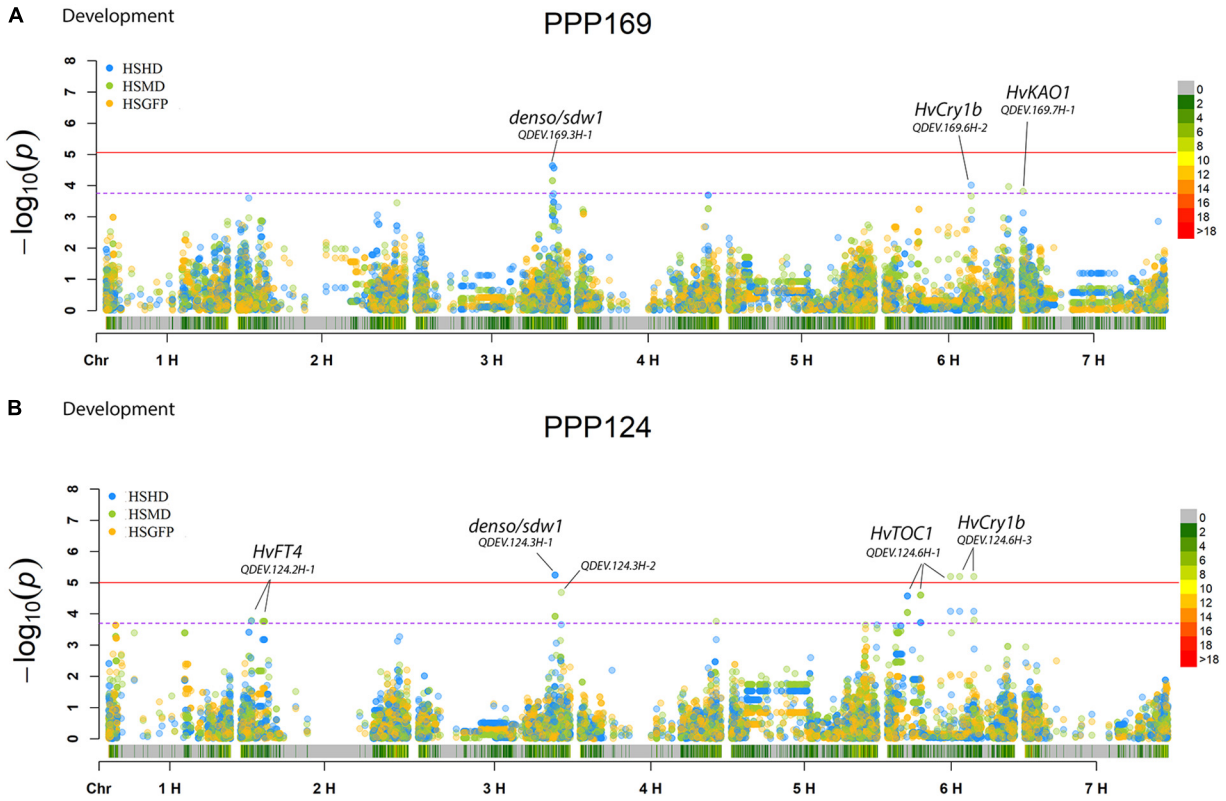

FIGURE 4 | GWAS results for the traits heat sum heading day, heat sum maturity, and heat sum grain filling period for panels **(A)** PPP169 and **(B)** PPP124, respectively. The Manhattan plots show significant associations (Bonferroni threshold in red, and the suggestive threshold in purple) between trait and marker, the x-axis shows the physical distance over all seven barley chromosomes. The bar under the x-axis shows the SNP distribution on each chromosome, where 0 ->18 depicts SNP density (the number of SNPs per 1 Mbp bin). The non-significant and significant associations are displayed as open and solid filled circles, respectively. The significance of the associations at values between these two extremes are displayed as circles with varying amounts of fill. The most significant values, located at the top, are displayed as solid filled dots. The significance of the associations at values between these two extremes is indicated by all other dots with varying amounts of fill. Relevant QTL names and putative loci are presented in the figure.

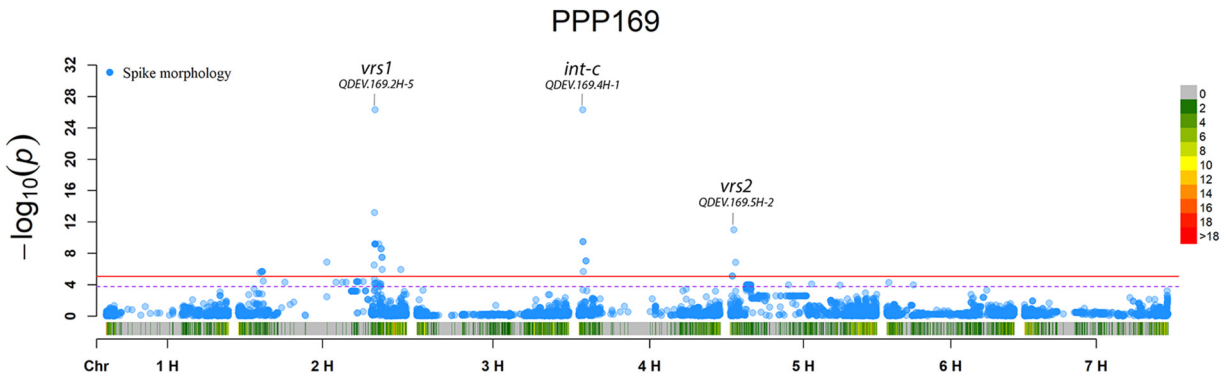

FIGURE 5 | GWAS results for spike morphology for panel PPP169. The Manhattan plots show significant associations (Bonferroni threshold in red, and the suggestive threshold in purple) between trait and marker, the x-axis shows the physical distance over all seven barley chromosomes. The bar under the x-axis shows the SNP distribution on each chromosome, where 0 ->18 depicts SNP density (the number of SNPs per 1 Mbp bin). The non-significant and significant associations are displayed as open and solid filled circles, respectively. The significance of the associations at values between these two extremes are displayed as circles with varying amounts of fill. The most significant values, located at the top, are displayed as solid filled dots. The significance of the associations at values between these two extremes is indicated by all other dots with varying amounts of fill. Relevant QTL names and putative loci are presented in the figure.

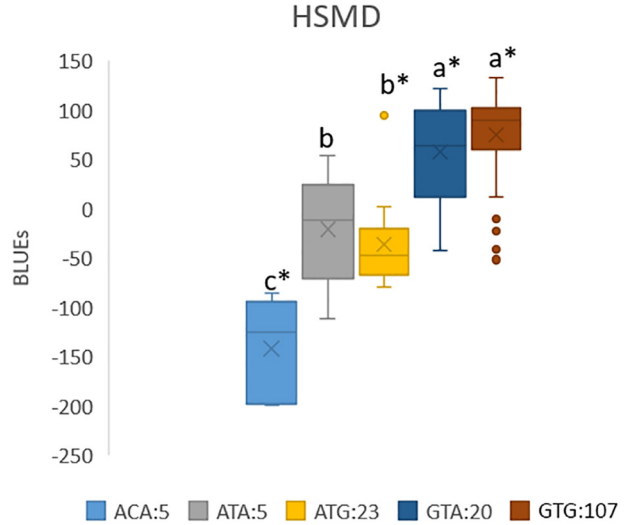

FIGURE 6 | Boxplot of allele combinations showing BLUEs for heat sum to maturity (HSMD) (°C) in the PPP169 panel. Allele combinations were based on three significant markers that passed the suggestive threshold value in the GWAS analysis. The effect of each allele combination for combinations with at least five observations (lines) was calculated based on BLUE values and the significance of the effects was tested using the lm () function in R. * marks significant combinations ($p \leq 0.05$). Allele combinations followed by the same lower case letter do not differ statistically.

earliness and height (Kuczyńska et al., 2014), and the fixation of QDEV.169.3H-1 in the two-rowed lines might reflect the historical breeding focus to shorten culm length in Nordic two-rowed lines (Dockter and Hansson, 2015).

Two QTL, QDEV.124.2H-1, and QDEV.124.7H-1, were associated with early vigor exclusively in the PPP124 panel (**Figure 2B**). QDEV.124.2H-1 included all significant markers detected on chromosome 2H in the PPP124 panel and was further confirmed by the identical allelic pattern observed for these markers. The most significant marker in QDEV.124.2H-1 with a position on 2HL is in close vicinity of *HvAP2*, previously shown to influence tiller number and plant height (Alqudah et al., 2016; Neumann et al., 2017).

For heading (PPP169/PPP124) and maturity (PPP124) we found a QTL located nearby the locus *HvCry1b* (**Figure 4**), which plays a role in the regulation of seed dormancy (Barrero et al., 2014), and has been reported as a putative heading associated gene (Alqudah et al., 2016).

When the most significantly associated markers for each trait were combined into allele combinations, we identified combinations with strong effect on earliness traits (**Figure 6** and **Supplementary Figure S3**). Especially noteworthy is the allele combination ACA which has a heat sum requirement 214 GDD below the most common allele combinations (GTG and GTA) in the Nordic barley gene pool (**Figure 6**). The ACA combination only occurred in five of the Icelandic lines (both two-rowed and six-rowed). In average, over all trials where maturity was scored, the difference in heat sum requirement to maturity equals 13.5 days shorter growth season. However, in the cool Icelandic conditions, the difference equals 19.4 days less from sowing to maturity compared with the most common allele combinations. In the sub-arctic environment, where harvest is typically done in September, this time can be the difference between a harvested mature crop and a crop destroyed by autumn frost and/or storms. The finding that the ACA allele combination was only found among Icelandic lines highlights the importance of selecting breeding lines in the target environment.

Phytohormone-Related Genes Associated With Straw Properties

For StL, we report a QTL (QDEV.169.3H-1) near the *denso/sdw1* locus on chromosome 3H in the PPP169 panel (**Figure 3A**), with an effect of 9 cm. The GA20 oxidase gene (*Hv20ox2*), involved in biosynthesis of gibberellic acid (GA), has been identified as a candidate for the *denso/sdw1* gene which could explain its effect on plant height (Jia et al., 2009). This QTL was, except for six lines, fixed among the two-rowed lines and therefore not detected in the GWAS analysis of the PPP124 panel (**Figure 3B**).

Another significant QTL (QDEV.169.2H-4), also with an effect of 9 cm, was found on 2H in the PPP169 panel. This is located close to the *HvDWF11* locus (Dockter et al., 2014), which has earlier been reported as a brassinosteroid-related gene in rice (Tanabe et al., 2005).

In the PPP124 panel three QTL, located on chromosomes 1H, 2H, and 5H, were found associated with StL (**Figure 3B**). The peak at 1H (QDEV.124.1H-2) did not correlate with known loci for height, but Alqudah et al. (2016) found a marker only 8kb distally with an effect on tiller number. The additional QTL, QDEV.124.2H-1, and QDEV.124.5H-1, identified for StL in PPP124 were previously reported to be associated with tiller number by Neumann et al. (2017). QDEV.124.5H-1 has been reported to associate with lodging (Tondelli et al., 2013).

Interestingly, for straw breaking, several significant marker-trait associations within QDEV.169.2H-3 were located nearby the region of the gene *HvGID2* (**Figure 3**; Marzec and Alqudah, 2018). In addition, a QTL, QDEV.169.5H- 5, was found close to the GA20 oxidase gene *HvGA20ox1* (Alqudah et al., 2016) was identified here to associate with straw breaking.

For lodging two QTL were found on 5HL (**Figure 3**), one of them, QDEV.169.5H-4, was located nearby a previously reported QTL for lodging (Tondelli et al., 2013). The relatively low significance of the associations with lodging and straw breaking could be explained by the difficulty in scoring these traits as we observed very little lodging or straw breaking the first 2 years. In year three, the nitrogen level was doubled in Denmark and Iceland, to promote lodging, with some success in Iceland but with less success in Denmark.

There was a general difference in the statistical strength of the associations between traits. Associations with earliness traits were weaker than associations with row type, early spring growth, and height. Row type had by far the strongest association. Early spring growth, measured as height of the foliage when plants had reached growth stages 31, 32, and 34 (Zadoks et al., 1974) was the second most significantly associated trait after row

type. This trait showed a very high correlation across locations and years, had a high heritability, and therefore potentially a smaller number of controlling loci compared with the flowering pathway. As earliness is known to be controlled by a relatively large and intricate network of loci (Blümel et al., 2015) whereas height is controlled by few loci (Kuczyńska et al., 2013), this suggests that simple inherited traits controlled by few loci were more easily detected than complex traits controlled by multiple loci. These findings are therefore a validation of the GWAS model used.

CONCLUSION

Although, most lines in our study showed a low degree of straw breaking we identified one allele combination, GGA, with a significantly higher rate of straw breaking (**Supplementary Figure S3**). This allele combination could be used to actively select against weak straw in the Nordic breeding programs.

The BLUE distributions showed a considerable difference between the two panels, with the PPP169 panel having a greater range of diversity for all traits. This is, at least for the earliness traits and the early vigor, most likely due to a small number of extremely early six-rowed barley lines from Iceland, that all headed earlier than 50 days in the field trials (see **Supplementary Figure S1**). The low number of these extremely early lines, only 3 such lines were included, made it hard to detect the effects of the underlying loci in the GWAS. Interestingly, the extremely early lines from Iceland, all carried the same allele at markers BK_12, BK_14, BK_15, and BK_16, all located within the *Ppd-H1* locus, different from the rest of the Nordic material. However, this allele combination was also found in the single two-rowed line of intermediate earliness from the United Kingdom. The fixed allele combination among the four *Ppd-H1* associated markers is therefore insufficient to explain the extreme earliness observed in the Icelandic material, and no other loci are found in the GWAS that could on their own explain the extreme earliness. Evidence does, however, suggest that a polymorphism at the markers 12_30894 and 12_30895, both located within the *HvFT1* locus, found in the Icelandic lines and not in the previously mentioned United Kingdom line might at least partly explain this observation. To further elucidate the genetics behind the unique agronomic performances of these extremely early lines a segregating multi-parent advanced generation intercross (MAGIC) population has been produced.

We here report the first GWAS of developmental traits focusing exclusively on Nordic spring barley from all five Nordic countries including both two- and six-rowed cultivars. Previous studies have found Nordic barleys to carry allelic diversity in many loci affecting early heading and early maturity (Tondelli et al., 2013; Loscos et al., 2014). This was confirmed in our study. In a few of the known flowering loci the pattern of allelic diversity is clearly different between row types, for example alleles for markers located in the *HvFT1* and *HvCEN* genes are fixed in the two-rowed lines but there is diversity among the six-rowed lines. Based on our results we could identify ideal allele combinations for regional adaptation to the unique day length and climate conditions in the extremely northern latitude, which could help push the margin for barley cultivation both in the north and possibly at other marginal areas.

AUTHOR CONTRIBUTIONS

AJ, MV, MA, LR, GB, JO, BG, RH, MG, and BE were involved in the planning and experimental design of the study. LK, RH, RA, MI, MJ, LR, TC, MG, and JH were managing the field experiments and phenotyping in field. JO, GB, and MG were managing the laboratory experiments. TB, MG, JO, ML, GB, and JHH performed data and statistical analyses. MG, TB, JHH, and ML wrote and critically reviewed the manuscript, made the figures and finalized the tables. All authors contributed to the discussion of the results and the editing and approval of the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2019.00542/full#supplementary-material

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

Paper II

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Crop Science

ORIGINAL RESEARCH ARTICLE

Crop Breeding & Genetics

Specific adaptation for early maturity and height stability in Icelandic spring barley

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Abstract

Cereal production in important growing regions is negatively influenced by climate change. This can be countered by expanding cereal production northwards in Scandinavia and Iceland, where today, barley (*Hordeum vulgare* L.) is primarily used as feed, as it rarely reaches malting quality. This study explores genetic factors underlying the ability of barley to mature fully in low temperature and long photoperiod. A panel of 84 spring barley lines were grown in controlled environments with different day lengths and temperatures, partially mimicking the target environment. The panel was screened for accumulated heat sum to heading, maturity, and height, all traits of importance for adaptation to the northern periphery. Subgroups with different stability and heat sum requirements were found, and day-length-neutral lines were identified. Height was temperature controlled, with lower temperature resulting in taller plants. The results were coupled to a genome-wide association study (GWAS). Despite the small panel size, the *Mat-a* locus was identified to have the strongest association with heat sum to heading; *Ppd-H1*, *Mat-a, FT1*, and *DHAR2* with heat sum to maturity; and *GA20ox1* with height. Early maturing lines with height stability have successfully been developed in Iceland, and this study confirms their performance in controlled environments for the first time. It provides insight to the mechanisms behind early maturity that will increase our ability to further adapt barley and other cereals to the northern climate. This will facilitate breeding work toward combining early maturity and height stability with traits such as quality, further enabling the northward expansion of grain production.

1 | INTRODUCTION

Barley (*Hordeum vulgare* L.) is the most widely adapted of the cereals (Ullrich, 2011) and, as such, is better suited to many marginal areas than, for example, wheat (*Triticum aestivum* L.). The environmental adaptation makes it the most important cereal crop in high latitude regions such as northern Scandinavia and Iceland (Nuttonson, 1957; Peltonen-Sainio

Abbreviations: BLUE, best linear unbiased estimate; GA, gibberellic acid; GDD, growing degree-days; GLM, general linear model; GWAS, genome-wide association study; HD, days to heading; HSHD, heat sum to heading; HSMD, heat sum to maturity; LDC, long day cold; LDW, long day warm; MD, days to maturity; MLM, mixed linear model; MLMM, multilocus mixed linear model; MTA, marker–trait association; NIL, near-isogenic line; PCA, principal component analysis; QQ, quantile–quantile; QTL, quantitative trait loci; SDC, short day cold; SDW, short day warm; SNP, single nucleotide polymorphism.

et al., 2011). Despite its adaptability, grain yield in the more northern regions still trails behind more favorable areas, with the average barley yield in Norway, for example, at 4.2 t ha^{-1} in 2017 vs. yields in regions such as Denmark and Germany with 6.0 and 6.9 t ha^{-1}, respectively (http://faostat.fao.org). Reports show stagnating yields in southern and central Europe (Schils et al., 2018), while yields in northern Europe are still rising (Moore & Lobell, 2015). Dawson et al. (2015) attribute this to effects of climate change, with the prediction that the impact will be even stronger in the future. Breeding of cultivars better adapted to local environments could potentially close the yield gap in northern latitudes. Research by a joint Nordic consortium for the promotion of plant breeding (Samnordisk planteforædling, 1992) showed that temperature is the climatic factor that mostly affects plant growth in northern Scandinavia. Iceland is at the margin of possible barley cultivation with a low temperature and a long photoperiod, the warmest month of the year averages 11 ˚C (Icelandic Meteorological Office). The growing season is relatively long in Iceland with sowing usually possible in the latter half of April and harvest in September (Hilmarsson et al., 2017). However, because of its maritime climate, the accumulated heat sum during the growing season is lower in Iceland than other regions at comparable latitudes (Martin et al., 2017) especially during the grain-filling period in late summer and early fall. The effects of climate change are particularly difficult to predict in Iceland where models range from an increase to even a slight reduction in temperature caused by the melting of Greenland ice cooling the ocean around Iceland (IPCC, 2018). One unambiguous effect of climate change is more extreme weather events (Trnka et al., 2014). Tall plants are prone to lodging after strong winds accompanied by heavy rainfall, thus leading to yield losses (Dockter & Hansson, 2015). The ability to withstand strong winds, occasionally in combination with heavy rainfall, is a crucial character for a stable yield in Iceland (Bragason, 1985). Experience from 40 yr of barley breeding and cultivar testing in Iceland (e.g. Hilmarsson et al., 2017) has pinpointed the most limiting factor in the northernmost areas of barley cultivation to be the ability to reach maturity in low temperature. The flowering time is also important, as a plant flowering too early may risk damage during anthesis because of late spring frosts, whereas flowering too late may risk that the crop does not fully mature before harvest (Bragason, 1985).

Genetic variation affecting the photoperiod response has enabled the successful expansion of barley cultivation from its origin in the Fertile Crescent to northern latitudes (Jones et al., 2008; Turner et al., 2005). Based on their response to altered day lengths, plants can be divided into three types: long-day responsive plants that flower in the spring under increasing day lengths, short-day plants that initiate flowering in response to shortening of the day in autumn, and day-length-neutral plants that flower irrespective of day length and take their cues from temperature (Andrés & Coupland, 2012).

Core Ideas

- Early maturity and height stability are key traits in adaptation of cereals to high latitudes.
- We investigated effect of day length and temperature on a panel of Nordic spring barley.
- Temperature had effect on plant height with few lines showing height stability.
- Heat sum requirement to maturity varied among the panel according to day length.
- Key MTAs found in a GWAS included *GA20ox1*, *Mat-a*, *Ppd-H1*, and *FT1*.

The progenitor of cultivated barley is a long-day responsive plant that flowers when day length exceeds a critical length but shows a substantial delay in flowering when grown under short-day conditions (Laurie et al., 1995), although, day-length-neutral plants have also been identified and genetically characterized (Gustafsson et al., 1971; Milec et al., 2013; Zakhrabekova et al., 2012). In northern latitudes (>60˚ N), spring sown barley is the predominant cereal crop, and it has been characterized by a photoperiod nonresponsive allele in the *Ppd-H1* locus delaying flowering in spring-sown barley plants to utilize the season with increased day length for vegetative growth, thereby increasing total yield (Sharma et al., 2020; Turner et al., 2005). A mutation in the *Mat-a* (synonyms *Eam8*; *ELF3*) gene has been attributed to enabling barley cultivation in northern Scandinavia and Iceland (Zakhrabekova et al., 2012). Spring barley plants carrying the mutated *mat-a* allele flowers earlier in long-day conditions (Faure et al., 2012; Zakhrabekova et al., 2012).

Plant height is an important trait in barley adaptation to windy and rainy environments since short and strong stems help to prevent lodging in addition to positively affecting the harvest index (Hay, 1995). Allelic variants of semidwarfing genes have been widely employed in modern barley breeding (Kuczyńska et al., 2013; Wang et al., 2014). Modern European barley cultivars generally depend on allelic variation in the *denso/sdw1* locus as their source of semidwarfing (Kuczyńska et al., 2013). Previously, it has been reported that the gibberellic acid (GA) responsive locus *denso/sdw1* influences both height and earliness in barley (e.g. Göransson et al., 2019; Jia et al., 2009).

Icelandic barley cultivars have shown extreme earliness in multilocation field trials from Bavaria (Germany) in the south to Iceland in the north, suggesting that lines selected in the low temperatures in Iceland have a lower heat sum requirement to heading and maturity (Göransson et al., 2019). Another observation from field trials is that many barley cultivars grow taller in Iceland than in other North European regions, such as Scandinavia, giving rise to speculation that the low temperature may affect the height of the plant (Göransson et al., 2019).

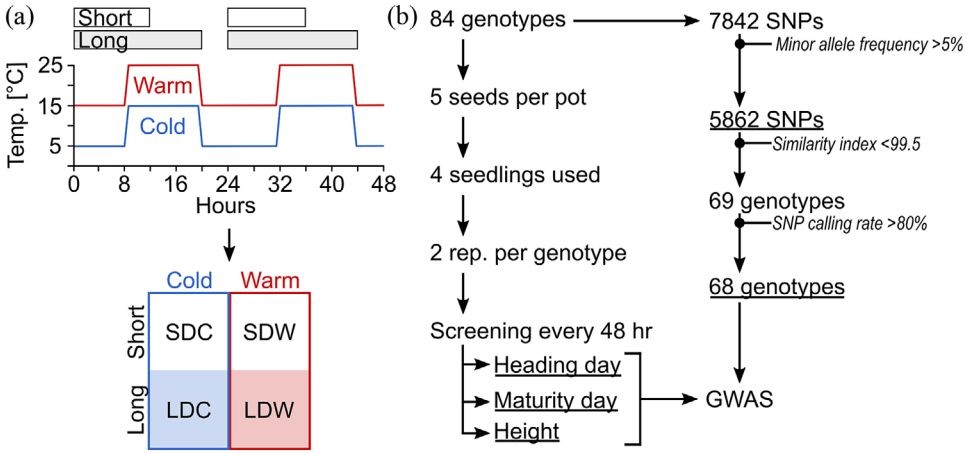

FIGURE 1 Experimental setup and overview of analysis. (a) Plants were kept at two day lengths, long day (20 h light/4 h dark) and short day (12 h light/12 h dark), each at two different temperature settings, cold (5–10 ˚C for 12 h, 15 ˚C for 12 h), and warm (15 ˚C for 12 h, 25 ˚C for 12 h), resulting in four different treatments: long day cold (LDC), long day warm (LDW), short day cold (SDC), and short day warm (SDW). (b) Eighty-four genotypes were selected for analysis and screened for three phenotypes: heat sum to heading, heat sum to maturity, and height. Each genotype was initially screened for 7,842 single nucleotide polymorphisms (SNPs) reduced to 5,862 SNP; after quality control the number of genotypes was reduced from 84 to 68 (see text for details)

The objectives of the current study are (a) to study effects of day length and temperature on the traits heat sum to heading, heat sum to maturity, and plant height on a panel of barley lines from Iceland and Scandinavia, and (b) to determine the quantitative trait loci (QTL) affecting the phenotypic differences.

2 | MATERIALS AND METHODS

2.1 | Plant material, growth conditions, and phenotyping

Twenty-seven two-rowed and 57 six-rowed spring barley cultivars and breeding lines of northern European origin were selected for analysis (Table 1). The lines were selected with the aim of representing diversity in heat sum requirements to maturity and day-length responses. The selection was based on previous results from performance in field trials (Nurminiemi et al., 1996), experience from the Icelandic barley breeding program (J. Hermansson and M. Göransson, unpublished results) and the results of a preliminary greenhouse study (M. Lillemo, unpublished results). Three near-isogenic lines (NILs) with allelic diversity at the *Mat-a* and *Uzu* loci were included along with their two-rowed mother cultivar Bowman to compare the phenotypes of known mutant lines with the Nordic population.

Seeds were sourced from (a) the original collection used by Nurminiemi et al. (1996) (these accessions were regenerated in greenhouse conditions prior to the experiment and later deposited at the regional gene bank, NordGen [www.nordgen.org]), (b) newly regenerated seeds from the Icelandic barley breeding program (Agricultural University of Iceland), (c) from the Byggbasis collection at Norwegian University of Life Sciences (regenerated in greenhouse prior to the experiment), and (d) 12 accessions were sourced from NordGen (Table 1). Five seeds per line were sown in 2-L pots with potting mixture (peat soil with clay and sand, Gartnerjord, Tjerbo, Norway). After emergence, four seedlings (or less if the germination was poor) were kept. No large deviations in plant emergence were observed except for two pots where no seeds germinated, and these are marked as 'NA' in the data set (Supplemental Table S1). The experiment had two replicates in each treatment, giving 168 pots in each of the four climate-controlled greenhouse chambers. The lines were grown for 180 d in four different environments in a climate-controlled greenhouse (Figure 1). The contrasting temperatures were chosen to approximately span the variation in growing season temperature for barley in the Nordic region in current and future climate (Børgesen & Olesen, 2011; Hilmarsson et al. 2017). The contrasting photoperiods of 20 and 12 h were chosen to enable detection of day-length responses (Kikuchi et al., 2012; Zakhrabekova et al., 2012). The treatments were (a) long day cold (LDC) with 20 h of light (illumination with high-pressure sodium lamps) and a recorded average temperature of 10 ˚C (~15/5 ˚C [day/night, each temperature period lasting 12 h]), (b) long day warm (LDW) with 20 h of light and a recorded temperature of 19.7 ˚C (~25/15 ˚C), (c) short day cold (SDC) with 12 h of light and a recorded temperature of 13.1 ˚C (~15/10 ˚C), and (d) short day warm (SDW) with 12 h of light and a recorded temperature of 19.9 ˚C (~25/15 ˚C). Temperature and humidity (maintained at constant 60%) were recorded continuously to monitor if the selected parameters were maintained

TABLE 1 Barley lines used in the experiment

Entry	Name	Row type	Seed source[a]	Accession number	Origin	Pedigree[b]	Release year[b]
1	'Brage'	6	Byggbasis	–	Norway	Lavrans//NK91650	2010
2	'Heder'	6	Byggbasis	–	Norway	SWN93162/Fager	2007
3	'Tiril'	6	Byggbasis	–	Norway	VoH91723/Arve	2004
4	'Edel'	6	Byggbasis	–	Norway	Tore/Verner	2002
5	'Helium'	2	Byggbasis	–	Denmark	Meltan/Delibes	2001
6	'Iver'	2	Byggbasis	–	Norway	Tyra/P-13	2001
7	'Marigold'	2	Byggbasis	–	France	UN97-5/UN1750	2009
8	'Iron'	2	Byggbasis	–	Denmark	Marnie/LP 813.6.98	2011
9	'Tyra'	2	Byggbasis	–	Norway	Sold/3/Birgitta/Mari//Gunilla	1988
10	'Trine'	6	SLU	NGB 6604	Norway	Lise × Clermont	1986
11	H3003	2	NordGen	NGB24716	Norway	Vada/203-7489//Jessica	–
12	'Lise'	6	Byggbasis	–	Norway	Asplund/DS295//Varde	1964
13	'Tunga'	6	SLU	NGB25777	Norway	Fræg//Juli/Rigel	1975
14	'Triumph'	2	SLU	NGB31409	Germany	Alsa/S 3170//11719-5913/Union/4/Diamant	1973
15	'Lavrans'	6	Byggbasis	–	Norway	Vera//Arve/H82009-1-3	1999
16	'Arve'	6	Byggbasis	–	Norway	Otra/Vigdis//Agneta	1990
17	HJA71384	6	SLU	NGB25765	Finland	Hankkija-673/Pomo	–
18	HJA77028	6	SLU	NGB24647	Finland	Eero mutant	–
19	Sigur_F	6	SLU	NGB25766	Faroe Islands	Landrace	–
20	'Tampar'	6	NMBU	NGB25785	Faroe Islands	Landrace	–
21	Is_046	2	SLU	NGB24648	Iceland	Mari/Tampar//Akka/Sigur-F	–
22	Vo_H_10660	6	SLU	NGB24651	Norway	Otra/Vigdis//Agneta	–
23	'Bode'	6	SLU	NGB25784	Norway	Pirkka/Nordlys	1978
24	'Fraeg'	6	SLU	NGB25783	Norway	Asplund/Maskin	1948
25	IGP_Fg_672_2_10_1	6	SLU	NGB25782	Norway	Fraeg mutant	1964
26	IGP_M_268	6	SLU	NGB25781	Norway	Fg-672-2-10-1/Dc-y-69b	1968
27	'Varde'	6	SLU	NGB25779	Norway	Asplund/Maskin	1941
28	'Yrjar'	6	SLU	NGB25778	Norway	Jarle/Varde	1975
29	HJA78045	6	SLU	NGB24657	Finland	Eero/Pomo//Potra	–
30	'Agneta'	6	SLU	NGB31405	Sweden	Asa/Frisia//Monte Cristo/4 x Edda II	1978
31	'Bamse'	6	SLU	NGB25775	Norway	2 x /IAsa/Frisial3/Monte Cristo/4 x Edda II	1981
32	'Jo_Silja'	6	SLU	NGB25774	Finland	Seger/Vega (6r)//Suvi	1979
33	'Arla'	2	SLU	NGB25770	Sweden	Maja/3/Hanna/Svanhals//Opal/4/Tammi	1962
34	'Akka'	2	SLU	NGB25769	Sweden	Monte Cristo/6 x Arla	1970
35	IPK_H2207	6	SLU	NGB24666	Norway	Lise/Paavo//Agneta	–
36	VoH2845	6	SLU	NGB24669	Norway	Bamse/4/Otra/3/Anital/Bonus/Varde	–
37	HJA78023	6	SLU	NGB24676	Finland	Olli/Hiproly//Kajsa	–
38	IGP_58	6	SLU	NGB25793	Norway	Domen/Fraeg	1968
39	IGP_H349220	6	SLU	NGB24677	Norway	Bode/Agneta	–
40	VoH2825	6	NordGen	NGB24681	Norway	Bamse/Yrjar	–
41	JO_1279	6	SLU	NGB25796	Finland	Otra/Etu	–
42	IGP_H349_10	6	NordGen	NGB24697	Norway	Bode/Agneta	–
43	HJA77061_Eero80	6	SLU	NGB25797	Finland	Olli/Eero	1985

(Continues)

TABLE 1 (Continued)

Entry	Name	Row type	Seed source[a]	Accession number	Origin	Pedigree[b]	Release year[b]
44	JO_1252	6	SLU	NGB24708	Finland	Varde/Otra	–
45	JO_1328	6	SLU	NGB24711	Finland	Suvi/Otra	–
46	HJA_77065	6	SLU	NGB24718	Finland	Etu/Pirkka	–
47	JO_1103	6	SLU	NGB25803	Finland	Varde//Opal/Perttu	–
48	JO_1310	6	NordGen	NGB24725	Finland	Jet/Ingrid	–
49	JO_1297	6	SLU	NGB24736	Finland	Suvi/Pirkka	–
50	JO_1184_Arra	6	SLU	NGB25808	Finland	Varde/Otra	1982
51	JO_1343	6	SLU	NGB24756	Finland	Varde/Otra	–
52	'Nairn'	2	AUI	–	Scotland	TRUMPF/HB-855-467-8	1984
53	Scots_Bere	6	AUI	–	Scotland	Landrace	–
54	247_1	6	AUI	–	Iceland	Arve/Hrutur	–
55	247_11	6	AUI	–	Iceland	Arve/Hrutur	–
56	Hrutur	6	AUI	–	Iceland	Arve//Nairn/VoH2825	–
57	'Kria'	2	AUI	NGB 16564	Iceland	Pernilla/Skegla	2004
58	'Skegla'	2	AUI	NGB 16565	Iceland	Pernilla/VoH2825//Pernilla	2002
59	291_13	6	AUI	–	Iceland	Arve/Skumur III	–
60	291_8	6	AUI	–	Iceland	Arve/Skumur III	–
61	294_12	6	AUI	–	Iceland	Arve/SjD918011//TIRIL	–
62	'Skumur_III'	6	AUI	–	Iceland	ARVE/SjD918011	–
63	SjD918011	2	AUI	–	Denmark	046/JO1328//Sepac	–
64	'Golf'	2	SLU	NGB25795	UK	Armelle/Lud//Luke	1983
65	'Pernilla'	2	SLU	NGB25768	Sweden	Birgitta/Mari//Gunilla	–
66	'Mari'	2	AUI	–	Sweden	Bonus mutant	1960
67	'Asa'	6	AUI	NGB 1487	Sweden	Dore/Wega	1949
68	'Asplund'	6	Byggbasis	–	Sweden	Selection from a mix of Primus I and 6-row barley	1910
69	'Maskin'	6	Byggbasis	–	Norway	Line selection from the landrace Bjorneby	1918
70	'Gunilla'	2	Byggbasis	–	Sweden	Birgitta x Opal/Vega//Gull mutant 44-3	1973
71	'Skumur_I'	6	AUI	–	Iceland	ARVE/SjD918011	–
72	'Teista_II'	2	AUI	–	Iceland	Sunnita/2//Akka/046	–
73	HJA_Hankkija673	6	SLU	NGB25807	Finland	Otra/Paavo	1973
74	JO_1315	6	NordGen	NGB24688	Finland	Tammi mutant	–
75	'Elmeri'	6	Byggbasis	–	Finland	–	2009
76	'Sepac'	2	AUI	–	Denmark	SEWA/PF-52296	1990
77	'Amalika'	2	AUI	–	Denmark	–	–
78	'Morex'	6	NordGen	NGB 23015	USA	Cree/Bonanza	1978
79	'Mona'	2	NordGen	NGB 1499	Sweden	(Mildew resistant line of Mari) × Monte Cristo	1970
80	'Barke'	2	NordGen	NGB 16758	Germany	Libelle/Alexis	1996
81	'Bowman'	2	NordGen	NGB 22812	USA	Klages//Fergus/Nordi /3/ND1156/4/Hector	1984
82	Bowman_NIL_Eam8_w	2	NordGen	NGB20574	USA	Bowman mutant	–
83	Bowman_NIL_Erectoides_o_16	2	NordGen	NGB 22114	USA	Bowman mutant	–
84	Bowman_NIL_Uzu1	2	NordGen	NGB20787	USA	Bowman mutant	–

[a]Seeds were sourced from (1) the original collection used by Nurminiemi et al. (1996) stored at the Swedish University of Agricultural Sciences (SLU), (2) newly regenerated seeds from the Icelandic barley breeding program at the Agricultural University of Iceland (AUI), and (3) from the Byggbasis collection at Norwegian University of Life Sciences, and (4) NordGen.

[b]References for the pedigree data and release year were Nurminiemi et al. (1996), NordGen database (www.nordgen.org/sesto), and information from breeders and breeding companies.

(Supplemental Figure S1). The short-day treatment was secured by automated mechanical covering of the plants with dark curtains to effectively provide darkness during night, as the greenhouse chambers could otherwise have been exposed to daylight or light from neighboring chambers with lamps. The actual temperature in each climate-controlled greenhouse chamber throughout the experiment was used in subsequent heat sum calculations.

Plants were supported with bamboo sticks and tied up to prevent falling over and were continuously watered on a daily basis to avoid any drought stress. The pots were fertilized one to two times per week using a 1.2 mg L^{-1} nutrient solution consisting of a 1:1 mixture of calcium nitrate and Yara Kristalon (9–11–30, 7 MgO + micro) from the two-leaf stage until the first spikes matured. All plants were treated twice during the grain-filling stage with 1.25 ml L^{-1} Forbel 750 (fenpropimorph, BASF) and 350 mg L^{-1} Confidor 70 WG (imidacloprid, Bayer) to prevent powdery mildew infections and thrips. In addition, a preventive treatment with a sulfur burner was maintained throughout the experiment to prevent mildew infections. The plants were screened every 48 h and the traits recorded were days to heading (HD), days to maturity (MD), height, and row type. Days to heading was registered as the day of ~2 cm of awn emergence of the second earliest plant in each pot, corresponding to Zadoks growth stage 49 (Zadoks et al., 1974), which is the growth stage best corresponding to the actual fertilization event in spring barley (Alqudah & Schnurbusch, 2013). Days to maturity was recorded when the peduncle of the earliest straw of the second earliest plant in each pot turned yellow, indicating that the phloem ceased to allocate sugars to the kernels, corresponding approximately to Zadoks growth stage 87 (Zadoks et al., 1974). We chose to consistently report the heading and maturity of the second earliest plant in each pot. This was a precautionary measure to rule out bias from potentially mixed-in seeds (which we did not observe, all pots showed a consistent development). In the low-temperature treatments, the peduncle remained green in some cases after yellowing of the spike. For those cases, MD was scored when the kernels became mature (growth stage 87–89) (Zadoks et al., 1974). Days to heading and MD were used to subsequently calculate the accumulated heat sum (growing degree-days, GDD) for both HD and MD based on the recorded temperature in each climate-controlled greenhouse chamber, with a baseline temperature of 0 ˚C. The accumulated heat sum to heading (HSHD) and heat sum to maturity (HSMD) were used in subsequent analyses. Height, the third trait of interest, was recorded upon the closing of the experiment and was recorded as the height of the straw in centimeters from the soil level to below the spike as an average across all spikes in the pot. For plants that did not head, no straw length was registered. In cases where flowering had not occurred at the closing of the experiment, the apical meristem was dissected and inspected to record whether it remained in a juvenile stage or had initiated into a reproductive phase. In order to obtain quantitative data on all lines, the nonheading plants were assigned a heading day later than the closing of the experiments (180 d), and maturity 30 d later (210 d), considering that all the lines had entered the reproductive phase. The artificial heading and maturity days are marked in Supplemental Table S1. Row type was scored as two- or six-rowed spikes and was subsequently used to validate (a) that no mix-ups had been made during sowing and (b) that the data analysis worked, as peaks would be expected at the *Vrs1* and *Int-c* loci controlling the row type (Lundqvist et al., 1997) (Supplemental Table S6).

2.2 | DNA isolation and genotyping

Leaf tissue (~50 mg fresh leaf) was sampled around growth stage 13 (Zadoks et al., 1974). The leaf tissue was homogenized using a Tissuelyser (Retsch) and DNA extracted using a standard protocol with the DNeasy extraction kit from Qiagen. Approximately 50 ng μl^{-1} of DNA from each sample were submitted to Trait Genetics, Germany, for genotyping using the 9K iSelect single nucleotide polymorphism (SNP) chip from Illumina (www.illumina.com), which contains 7,842 SNP markers (Comadran et al., 2012). A total of 5,862 markers were polymorphic at >5% minor allele frequency level and their physical positions (in base pairs) on the barley reference genome (Mascher et al., 2017) were retrieved using the online tool BARLEYMAP (Cantalapiedra et al., 2015). The similarity index was calculated using the function mcor (pairwise complete correlations) in R Studio (RStudio Team, 2020), with a threshold of 0.99. Fifteen lines had a similarity index >99.5 with one or more lines and were thus excluded from genetic analyses (Supplemental Table S2). These lines were still included in phenotypic data as their relatedness could in many cases be explained by their recent pedigree. One line had a SNP calling rate <80% and was excluded from genetic data analyses. Thus, in all, 68 lines remained in the genetic data analyses (Figure 1b).

2.3 | Data analysis

The traits showed a normal distribution, and Pearson correlations between traits and environments were calculated using package corrplot (Wei & Simko, 2017) in R Studio (RStudio Team, 2020). Analysis of variance was calculated on randomized complete blocks using META-R v. 6.0 (Alvarado et al., 2016).

The model $Y_{ijk} = \mu + \mathrm{Env}_i + \mathrm{Rep}_j\left(\mathrm{Env}_i\right) + \mathrm{Gen}_k + \mathrm{Env}_i \times \mathrm{Gen}_k + \varepsilon_{ijjk}$ was used.

where Y_{ijk} is the trait of interest, μ is the overall mean effect, Rep_j is the effect of the jth replicate within the ith environment, Gen_k is the effect of the kth genotype, $\text{Env}_i \times \text{Gen}_k$ is the effect of the environment × genotype interaction, and ε_{ijjk} is the effect of the error associated with the ith environment, jth replication, and kth genotype. Environment and genotype were considered to be fixed effects and the best linear unbiased estimates (BLUEs) were analyzed as values of the two replications in each environment. Fisher's test was used to calculate differences between the treatments and genotypes (Minitab 17 Statistical Software, 2010). Broad-sense heritability for the traits in each respective treatment was calculated using META-R v. 6.0 (Alvarado et al., 2016). A population structure within a given set of barley genotypes can cause false positives in associations between markers and traits. The software STRUCTURE v. 2.3.4 (Pritchard et al., 2000) was used to predict the most likely number of subpopulations (K), with Markov chain Monte Carlo set to 9,999 burn-in phases and 9,999 iterations run 10 times for each simulated number of K between 1 and 12. Structure Harvester v.0.6.94 (Earl & von Holdt, 2012) was used to determine the most likely K using the method of Evanno et al. (2005). Principal component analysis (PCA) was performed using the genotype data to further explore population structure. To find marker–trait associations (MTAs) a genome-wide association study (GWAS) was performed using the R-package Genome Association and Prediction Integrated Tool (GAPIT v.3.0) (Lipka et al., 2012) following the methods in Göransson et al. (2019). Kinship matrices were constructed following the van Raden method incorporated in GAPIT. In subsequent mixed linear model (MLM) analyses (Yu et al., 2006; Zhang et al., 2010) the kinship matrix, K-values, and PCA eigenvalues were used to account for population structure. To select the optimal model, four single-locus and one multilocus models were tested: (a) general linear model (GLM) without a population structure, (b) MLM using van Raden kinship, (c) MLM using van Raden kinship and Q-values from the STRUCTURE analysis, (d) MLM with van Raden kinship and eigenvalues from the PCA, and (e) multilocus mixed linear model (MLMM) using van Raden kinship. Quantile–quantile (QQ) plots were constructed by comparing expected and observed $-\log(p)$ values (Supplemental Figure S2). Models were evaluated based on the QQ plots (large deviations from the expected distribution mean that the model does not fit the data) to select the most appropriate model for the population. Bayesian information criterion values were zero for eigenvalues from the PCA and hence these were excluded from the model evaluation. Out of the tested models and for all traits except spike morphology (two- vs. six-rowed phenotypes), the MLMM gave the strongest associations and was best in terms of correcting for both false positives and false negatives. For row type, the best model was MLM using van Raden kinship and Q-values. The MLMM is generally better suited to handle quantitative traits controlled by many loci in structured populations (Segura et al., 2012). Thus, for subsequent analysis, MLMM was used for HSHD, HSMD, and height. Manhattan plots showing positions of associated markers across the genome were constructed for each trait based on the MLMM results using the R package CMplot (Yin et al., 2020). Genome-wide association study was performed for each environment and trait based on the calculated BLUEs. A cut-off value of $-\log(p) \geq 3$ was used as the significance level for the association analysis (Li et al., 2012). Zhang et al. (2019) have recently recommended this cut off in multilocus GWAS to balance between the high power and low false positive rate for MTAs. For allele effects across the environments output from the MLM was used, whereas allele effects for specific environments were calculated based on the BLUEs for the respective environment. The interval lengths of the QTL were decided by calculating the linkage disequilibrium between the most significant markers at each intra-chromosomal locus using TASSEL v. 5.2.31 (Bradbury et al., 2007) and evaluating the linkage disequilibrium plot based on r^2 value and D prime, with threshold values of approximately 0.7 and 0.8, respectively. Barleymap (Cantalapiedra et al., 2015) and Barlex (Colmsee et al., 2015) was used to search for putative loci near the significant MTAs, with an approximate search window of <5 Mpb from the SNP marker. Allele combinations for MTAs with previously known earliness loci were tested for significance between groups (confidence interval 0.95) using ANOVA and Tukey's test, using the RStudio (RStudio Team, 2020) packages emmeans (Lenth, 2020), multcomp (Hothorn et al., 2008), and boxplots were constructed using the package ggplot2 (Wickham, 2016). Stability plots obtained from the genotype × environment analysis with R (GEA-R) v.4.1 (Pacheco et al., 2016) software were used to visualize the CV plotted against the BLUEs across environments trait wise for each genotype. The model used randomized complete blocks run as a linear regression model. All environments were included in the stability models for HSHD and HSMD, whereas for height the SDW environment was excluded because of missing data for the trait. A phenotype stable under different environments will lead to a lower CV value, while a higher CV suggests a stronger environmental effect in one or more environments. Figures 1 and 7 were created using Inkscape (https://inkscape.org).

3 | RESULTS

3.1 | ANOVA

The ANOVA showed the environment to be of largest effect in all traits (Supplemental Table S4). For HSHD and HSMD, the environmental effect accounted for >60% of the variation, whereas for height, there was only a small difference in

effect between the environment and the genotype effect (45 and 44% of the total variation, respectively). For all traits, the genotype × environment interaction was smallest (but significant for all traits). The data from all four environments were significantly positively correlated among each trait, the range of r values were 0.55–0.82, 0.24–0.65, and 0.4–0.86 for HSHD, HSMD, and height, respectively (Supplemental Figure S3). All four treatments had significant correlations between HSHD and HSMD (range of r values 0.32–0.94), whereas height was poorly or not correlated with HSHD and HSMD.

3.2 | Earliness

The heat sum requirement to heading showed a clear variation in plant phenotypes affected by the environments, ranging from long-day-responsive lines, which headed earlier in long-day conditions to lines with day-length neutrality that headed irrespective of day length (Figure 4). The broad-sense heritability (H^2) for HSHD were 0.87, 0.90, 0.80, and 0.80 for LDC, LDW, SDC, and SDW, respectively (Supplemental Table S3). Eleven lines, including both row types, proved to be day-length neutral for HSHD (Figure 2), two of these were NILs of cultivar Bowman with known mutations at the *Mat-a* locus (Bowman NIL Eam8.w and Bowman NIL Erectoides o.16). The other nine were cultivar Mari and eight other Nordic spring barley lines ('Teista II', IS-046, HJA77061(Eero80), 'Tyra', 'Iver', 'Mona', H3003, and HJA77028) all of, which have Mari in their pedigrees. Six lines did not reach the heading stage in SDW (Figure 2), with a dissection at the closing of the experiment showing that all of them had altered the meristem into the reproductive stage but had stagnated and not proceeded to bolting. This was seen in the Swedish cultivar Asplund and five others, which all had Asplund in their pedigrees. When the two long-day environments were compared, the HSHD was consistently higher in LDW than in LDC (Supplemental Figure S4). A stability plot of the phenotype data (Figure 3a) revealed a grouping of the day-length-neutral lines in the lower left corner. The lines that did not head in SDW grouped in the top right corner. A group of early stable lines grouped in the lower left square above the red marked lines (line 55, 247_11; line 58, 'Skegla'; line 57, 'Kria'; line 54, 247_1; line 56, Hrutur; and line 71, 'Skumur I') where all were selected in Iceland, except for one line from the Faroe Islands (line 20, Tampar) and one line from Finland (line 37, HJA78023).

Heat sum to maturity largely followed the pattern of the HSHD (Figure 2), meaning that HSMD was relatively constant irrespective of ambient temperature. However, there was a significant difference between the two long-day treatments ($p = .0068$), where a subgroup of primarily Icelandic lines showed a contrasting heat sum requirement from most other lines; instead of requiring a lower accumulated heat sum in LDC (as most lines) they required a higher heat sum in LDC (Supplemental Figure S4). Broad-sense heritability for HSMD was 0.70 in LDC, 0.90 in LDW, 0.81 in SDC, and 0.89 in SDW (Supplemental Table S3). A stability plot based on the phenotype data (Figure 3b) revealed a grouping of the day-length-neutral lines in the lower left corner. The lines that did not head in SDW grouped in the top right corner. A group of early stable lines grouped in the lower left square above the red marked lines were all were selected in Iceland (line 55, 247_11; line 58, Skegla; line 57, Kria; line 54, 247_1; line 56, Hrutur) or the Faroe Islands (line 20, Tampar).

3.3 | Height

Temperature had a large effect on the average height (Figure 2; Supplemental Figure S4). Most of the lines had the tallest plants in LDC followed by SDC, LDW, and finally SDW. The broad-sense heritability for height was 0.95 in LDC, 0.96 in LDW, 0.89 in SDC, and 0.91 in SDW, (Supplemental Table S3). The difference in height between cold and warm and long and short day, respectively (Figure 2c) illustrated how most lines responded to temperature by expressing taller phenotypes in cold temperature vs. warm temperature irrespective of day length ($p \leq .001$). A few lines, however, had a different pattern, with the three extremely early Icelandic lines, 247_11, 247_1, and Hrutur, consistently producing the tallest plants in the short-day treatments (Figure 2c). The height effect was also visible on the spikes in these three lines, producing a larger inflorescence in short-day treatments (data not shown). The line JO_1279 (also known as cultivar Nord) had the most extreme temperature response in long-day environments, where it almost doubled its height in LDC compared with LDW (Supplemental Figure S4). A barley NIL, with a mutation at the semidwarf locus *Uzu*, was included in the panel, as it has previously been reported to be responsive to variations in temperature (Dockter & Hanssson, 2015). In the current study, the stability plot for height (Figure 3c) indicates that line 84, Bowman NIL Uzu1, had the largest CV, which confirms the strong response to temperature. Several lines in the lower end of the graph had very low CV (line 20, Tampar; line 26, IGP_M_268; and line 72, Teista II), indicating height stability throughout the environments.

3.4 | Population structure and GWAS

The PCA and the STRUCTURE analyses both showed a similar pattern of population structure (Figure 5; Supplemental Table S5) with the 57 six-rowed lines forming one cluster and the 27 two-rowed lines forming three clusters. The Bowman-derived NILs along with their mother cultivar Bow-

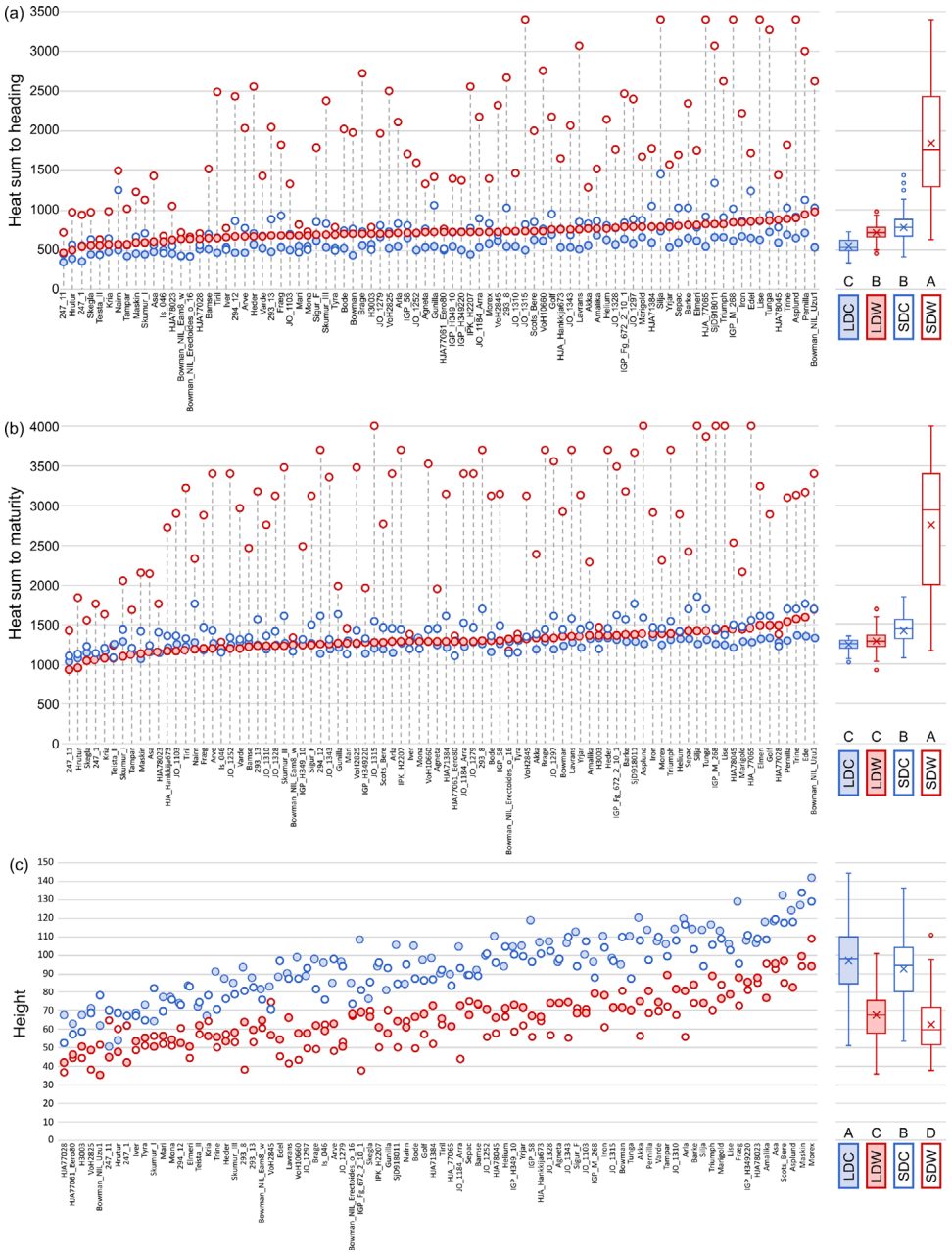

FIGURE 2 (a) Heat sum to heading (HSHD), (b) heat sum to maturity (HSMD), and (c) height for 84 spring barley lines grown in four environments: long day cold (LDC; 20-h day length and 15/5 ˚C, day/night; filled blue circles), long day warm (LDW; 20-h day light and 25/15 ˚C; filled red circles), short day cold (SDC; 12-h day length and 15/10 ˚C; open blue circles), and short day warm (12-h day length and 25/15 ˚C; open red circles). In (a) and (b), the genotypes tested are ordered from left to right based on the heat sum requirement under long day warm conditions, and in (c) they are ordered from left to right based on overall mean height from all environments. Fischer's least significant difference is indicated in the box plots, where means that do not share a letter are statistically significant

man formed one isolated cluster, and the remaining two-rowed lines formed two less distinct clusters. All day-length-neutral, two-rowed lines clustered together except for the two day-length-neutral NILs.

The GWAS analysis revealed 80 significant MTAs resulting in 21 QTL (Figure 6; Supplemental Table S6). For HSHD, eight significant MTAs were found; the most significant was in QDLT.1H.3, near (0.7 Mbp) the *Mat-a* locus, which was also the only one that showed effect in all four environments (Figure 7; Supplemental Table S6). This effect corresponded to 67 GDD, equal to 7 d in the cold treatments (or 3.5 d in the warm treatments). For HSMD, nine MTAs had a $-\log(p)$ value above the threshold (Figure 6b), half of which coincided with HSHD in one or more of the environments (Figure 7; Supplemental Table S6), but no significant association was consistent for all environments for the HSMD trait

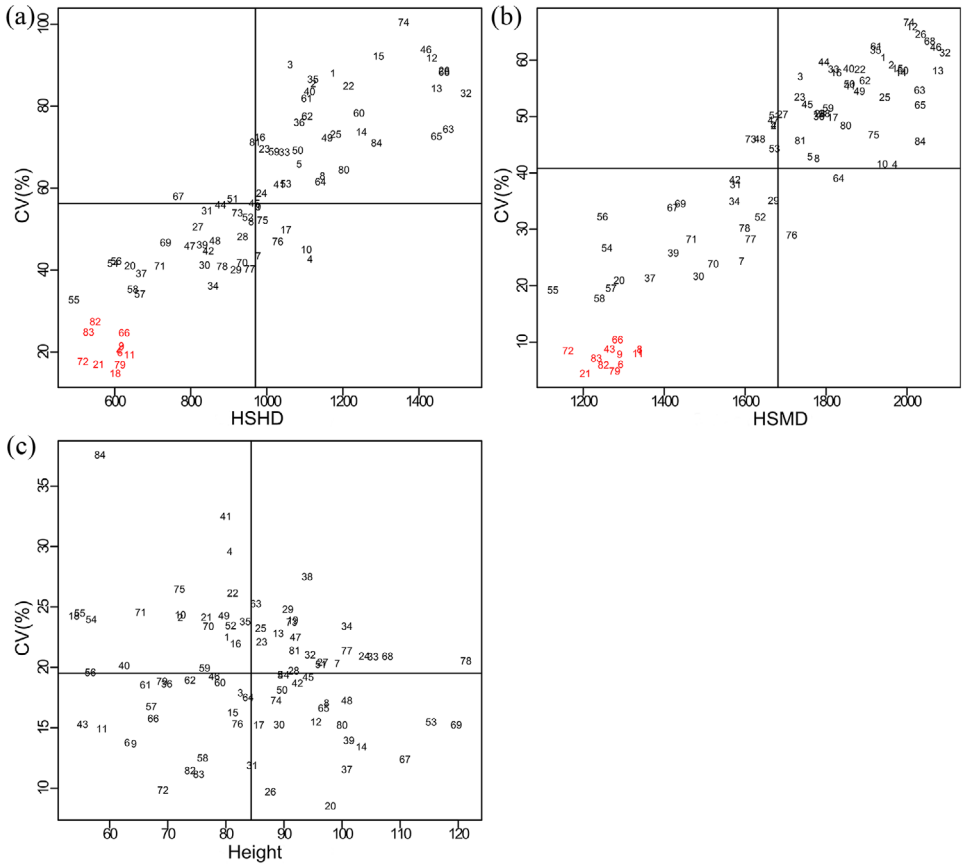

FIGURE 3 Stability plots showing the three phenotypic traits, (a) heat sum to heading (HSHD), (b) heat sum to maturity (HSMD), and (c) height plotted against the respective coefficient of variation calculated from the BLUE values from four environments: long day cold (LDC; 20-h day length and 15/5 ˚C, day/night), long day warm (LDW; 20-h day light and 25 /15 ˚C), short day cold (SDC; 12-h day length and 15/10 ˚C), and short day warm (12-h day length and 25/15 ˚C) (a) and (b), and the three environments LDC, LDW, and SDC in (c). The genotypes are plotted as numbers (1–84), the names of the corresponding genotype numbers can be found in Table 1. In (a) and (b), the 11day-length neutral lines are color coded in red

(Figure 7). In LDC, the most significant MTA was at QDLT.2H.1, physically located in the *Ppd-H1* gene, which had an effect of 140 GDD in LDC. In LDW the most significant MTAs for HSMD were found near the locus for *DHAR2* (0.3 Mbp), *Mat-a* (1.4 Mbp), and *FT1* (2.4 Mbp) (Figure 6b; Supplemental Table S6) with effects of 202, 175, and 50 GDD, respectively. For height, six significant associations were found, with SNPs located close to five known genes: *GA20ox1* (0.3 Mbp), *FTL5* (2.4 Mbp), *ARF19* (1.0 Mbp), *ARF4* (7.5 Mbp), and *EF-1a* (0.5 Mbp) (Figure 6c). The most significant association was found on chromosome 5H, located near (0.3 Mbp) the *GA20ox1* locus, which was also the only MTA for height that was consistent in all environments (Figure 7). The effect of the *GA20ox1* locus on height was 21 cm in LDC.

Only two MTAs for the three traits analyzed had an effect in all environments, for height, an MTA near *GA20ox1* (0.3 Mbp) and for HSHD near *Mat-a* (1.4 Mbp) (Figure 7), while a single MTA, found near the *denso/sdw1* locus (3.5 Mbp), had an effect in three different environments for HSHD. *Ppd-H1* (MTA located in the gene) was found in LDC for both HSHD and HSMD (Figure 7). An allele combination for the MTAs in the *Ppd-H1* (BK_16; 489 bp), and near the *Mat-a* (SCRI_RS_158298; 1.4 Mbp), and *FT1* (SCRI_RS_172761; 2.4 Mbp) loci explained 186 GDD for HSMD in the LDC environment, which corresponds to a maturity time 19 d earlier than the average (Supplemental Figure S5).

4 | DISCUSSION

The Icelandic environment poses challenges to crop production by the low temperature during the growth season. An extreme earliness in Icelandic barley lines has previously been reported (Göransson et al., 2019). The current study, for the first time, confirms the extreme earliness in a panel of barley lines developed for northern latitudes in controlled environments. It provides an important insight into the genetic mechanisms behind this early maturity and height stability that will enable a further expansion of cereal production northward. In this study, phenotypic data show that the heat sum

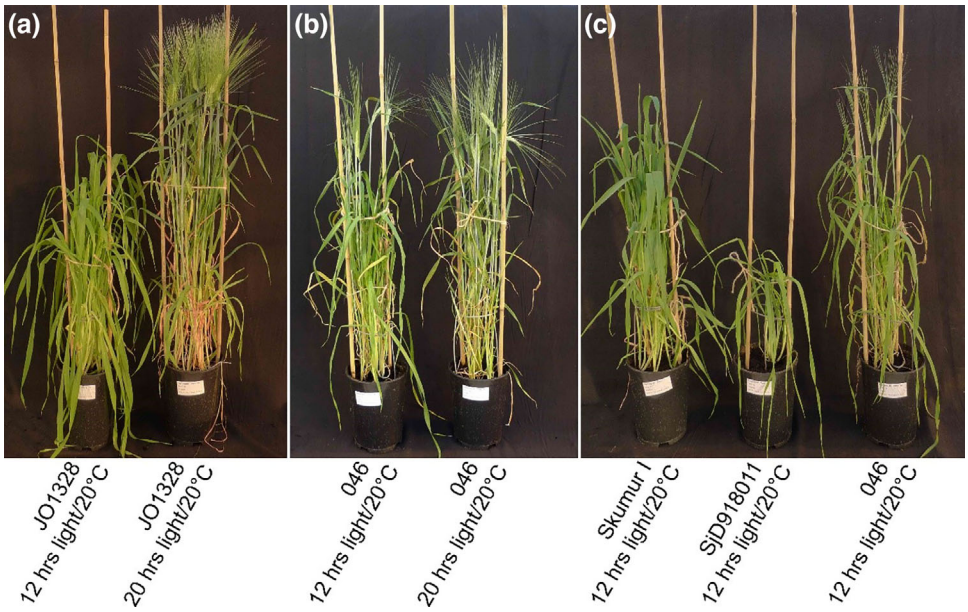

FIGURE 4 Examples of phenotypic differences in photoperiod response for plants grown in two environments: long day warm (LDW; 20-h day light and 25/15 ˚C) and short day warm (SDW; 12-h day length and 25/15 ˚C). (a) JO1328 growing in SDW (left) and LDW (right); (b) IS-046 growing in SDW (left) and LDW (right); (C) the mid-plant SjD918011 had severely delayed heading and stunted growth in SDW, whereas the left plant 'Skumur I' had delayed heading but normal growth, and the right plant, 046, was day-length neutral. All plants photographed 48 d after sowing

requirement to heading is consistently lower in LDC than in LDW for all lines ($p \leq .001$) (Figure 2a). Eleven genotypes were day-length neutral, of these two were NILs of cultivar Bowman with allelic variation in the *Mat-a* locus and nine Nordic genotypes, all of which descended from the induced mutant cultivar Mari, released in 1960 as an X-ray irradiated mutant of the Swedish cultivar Bonus (Gustafsson et al., 1971). Mari has been shown to carry a mutated allele in the *Mat-a* locus (Faure et al., 2012; Zakhrabekova et al., 2012). The locus with the strongest association to HSHD was *Mat-a* (Figure 6a; Supplemental Table S6), a locus known to infer day-length neutrality and previously proposed to infer adaptability to barley in diverse environmental conditions ranging from high altitudes in tropical regions to high latitude regions (Zakhrabekova et al., 2012). The locus was not found to be associated with heading in a previous study with similar material in field conditions (Göransson et al., 2019). In this study, however, with more contrasting environmental conditions, it was shown that nine of the Nordic barley genotypes included in the study indeed were day-length neutral, and all of them carried the same allele in the MTA for *Mat-a*. However, out of the 84 lines included, a total of 42 carried the same SNP allele, so it can be concluded that the MTA with the highest $-\log(p)$ value (SCRI_RS_158298) is not diagnostic for the day-length neutrality. A wider network of alleles is clearly at play, which is to be expected for a complex trait like heading. There was no clear pattern in heat sum requirements between cold and warm treatment under short-day conditions. The lines combining earliness with stability to environmental conditions (Figure 3a) had either a day-length-neutral allele in the *Mat-a* locus or were selected in Iceland.

Maturity in low temperature is the most important breeding goal in a cool maritime subarctic climate such as Iceland. There is often a long period during the grain filling period where temperatures slowly decline from the peak of 11 ˚C in July and August down to an average temperature of just above frost mark before harvest (Icelandic Meteorological Office). The panel showed three significantly different subgroups for relative HSMD in the long-day environments ($p \leq .001$): One group that followed the pattern for HSHD that is with a lower heat sum requirement in LDC than in LDW, one group with a similar heat sum requirement, and one group with a higher heat sum requirement in LDC than in LDW (Supplemental Figure S4B). Incidentally, the lines in the last group were largely the same as those showing the highest stability in height between LDC and LDW (Supplemental Figure S4). These lines, which are mostly Icelandic, have been selected in an environment similar to the LDC environment. The mean heat sum requirement of the first six lines in Supplemental Figure S4B were 1,159 GDD. This corresponds well with previously reported heat sum requirements to HSMD for early Icelandic barley of 1,200 GDD (Ólafsson et al., 2007). Göransson et al. (2019) found HSMD in field conditions in Iceland to vary between 1,019 to 1,418 GDD with the mean of 1,294 GDD for a panel of 180 commercial barley breeding lines of Nordic origin, where the earliest lines were Icelandic. The time of maturity in Icelandic field conditions coincides for these early lines with the end

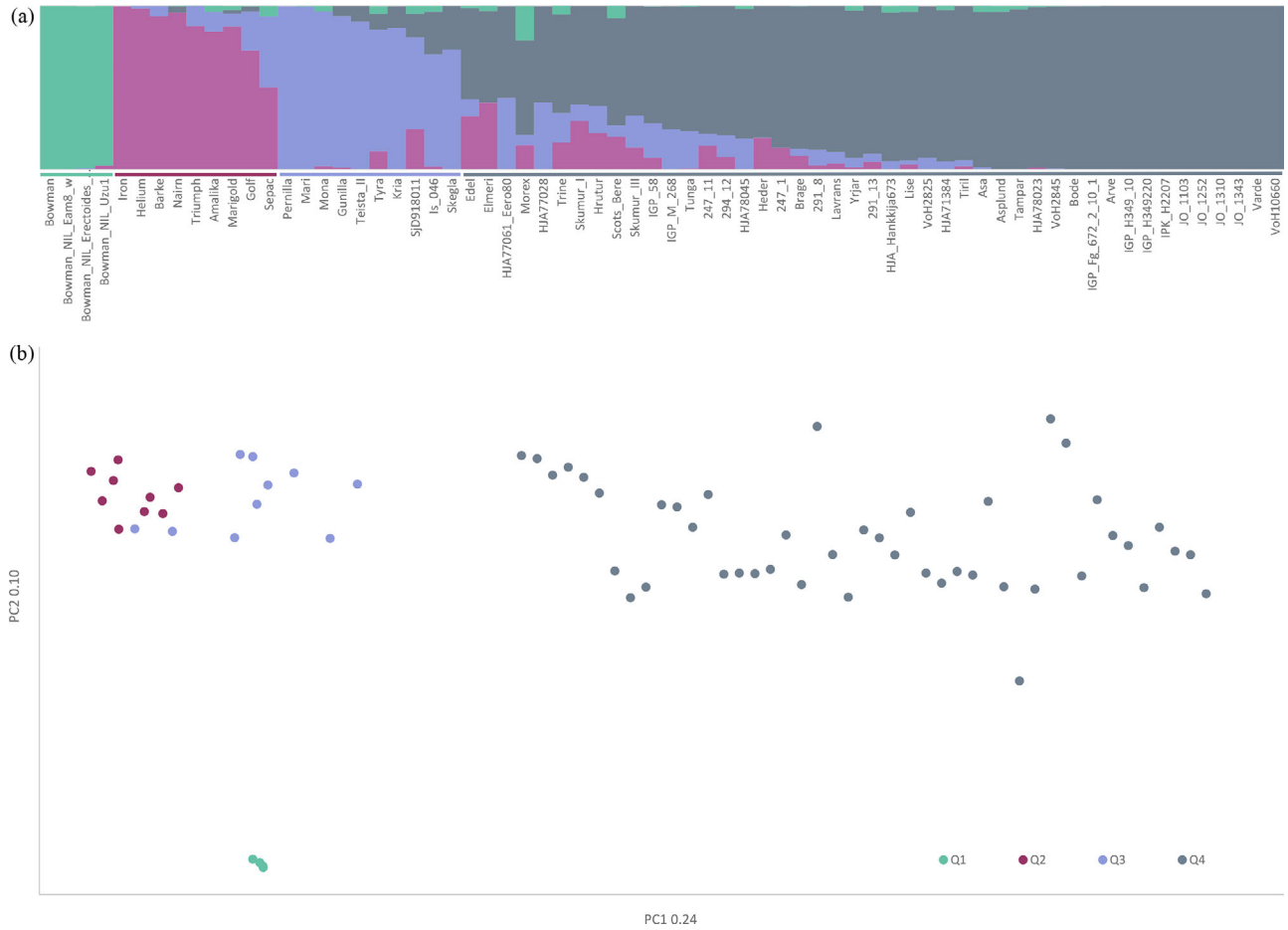

FIGURE 5 (a) Results of STRUCTURE analysis resulting in four subpopulations (color coded between the bar plot and the line names). (b) Principal component analysis (PCA) based on SNP markers grouped by STRUCTURE subpopulations (Q1–4). Q1, Q2, and Q3 where different constellations of two-rowed lines, and Q4 comprised of exclusively six-rowed lines

of the warmest summer period, in August. The reason why many Icelandic barley lines show a contrasting HSMD in cold vs. warm temperature (as seen in Supplemental Figure 4B) could be that they have been selected to head early, whereas less focus has been on their maturity time. The early heading could have led to the maturity occurring in the warmest season, leaving the early lines unexposed to the cold autumn temperatures to which later heading material had been exposed. In LDC, the strongest association with HSMD was with the *Ppd-H1* locus. The gene *Ppd-H1* is a major determinant of barley photoperiod response (Turner et al., 2005), with a dominant allele *Ppd-H1* most common in winter barley and a recessive *ppd-H1* allele more common in spring barley (e.g. 'Triumph' and most North European spring barley). The *ppd-H1* allele provides an adaptive advantage for the plant, which uses the spring to develop vegetatively instead of transitioning early to flowering in response to the increase in day length. The *ppd-H1* allele has previously been shown to be effectively fixed in Nordic spring barley (Göransson et al., 2019). However, the current panel had a higher frequency of the same lines that, in an earlier study, were shown to carry the sensitive allele but had been too few to enable detection through GWAS (Göransson et al., 2019). The allelic diversity in *Ppd-H1* seems to be interacting with other loci for the extreme earliness found in three Icelandic genotypes. An allele combination between *Ppd-H1*, *Mat-a*, and *FT1* with an average accumulated heat sum of 1,065 GDD for HSMD in the LDC environment (corresponding to 107 d) differed significantly ($p \leq .001$) from the overall mean of 1,251 GDD (corresponding to 125 d in LDC). We speculate whether the adaptation for low heat sum requirement to maturity involves GA regulation, and hypothesize that the earliest lines lack temperature sensitivity. Boden et al. (2014) found that the loss-of-function mutant of *Mat-a* has an increased expression of *FT1*, which was activated by the *GA20ox2* regulation of GA. Composite crosses maximizing the allelic diversity for the main earliness genes (especially the ones controlling the GA signaling pathway) could potentially create populations where this can be studied in more detail. It is interesting that *CEN*, a well-known earliness locus, did not show up in the GWAS. However, other studies have reported that a single allele of the *CEN* locus is virtually fixed in European spring barley (Tondelli et al. 2013;

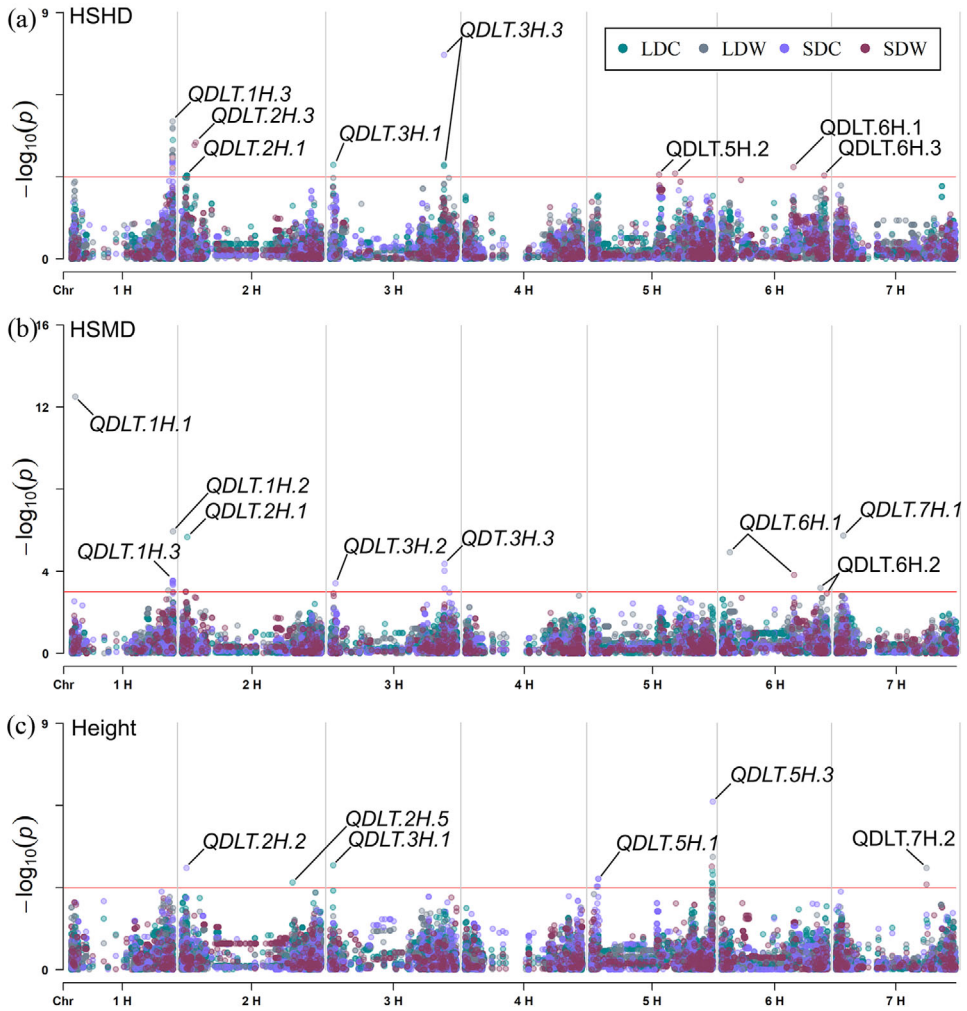

FIGURE 6 Manhattan plots showing significant associations for the traits (a) heat sum to heading (HSHD), (b) heat sum to maturity (HSMD), and (c) height in four environments: long day cold (LDC; 20-h day length and 15/5 ˚C, day/night), long day warm (LDW; 20-h day light and 25/15 ˚C), short day cold (SDC; 12-h day length and 15/10 ˚C), and short day warm (12-h day length and 25/15 ˚C). Quantitative trait loci are indicated and alternative putative loci can be found in Supplemental Table S6

Fjellheim et al., 2014; Göransson et al., 2019), which could explain why it was not found in the GWAS of the currently reported panel.

The study found that height varied mostly as a result of temperature and not day length. In low temperature, the plants grew taller (Figure 2c). However, a subset of lines of primarily Icelandic and Faroese origin showed a relative stability in height between the LDC and LDW environments. The phytohormone GA influences height and this has been used in barley breeding programs since the Green Revolution by incorporating semidwarf genes, which downregulates GA production (Xu et al., 2017), thus leading to shorter plants that can tolerate higher input of fertilizer without lodging. In Europe and North America, allelic variation at the *denso/sdw1* locus is most widely used to produce semidwarfing varieties (Jia et al., 2009). Allelic variation at the *denso* locus originating in the Danish cultivar Abed Denso and the Czech mutant cultivar Diamant, is allelic to the *sdw1* allele originating from the Norwegian cultivar Jotun (Jia et al., 2009). The *denso/sdw1* locus acts as a suppressor of GA and has been shown to be an ortholog of the rice *sd1* locus coding for the *GA20ox2* gene (Jia et al., 2009). Sakata et al. (2014) found that rice carrying the *GA20ox2* allele responsible for the *sd1* mutant showed hypersensitivity to low temperature for pollen development (which is regulated by the same semidwarf gene, *sd1*, that regulates plant height), which resulted in a disruption of the GA downregulation. The *GA20ox2* gene has a homologue, the *GA20ox1* gene, which has been reported to compensate for reduced function of *GA20ox2* (Xu et al., 2017). An MTA at the *GA20ox1* (*denso/sdw1*) locus was found to be significant in all environments, explaining an effect of 21 cm in LDC. All plants of the subset of lines showing height stability had the same *GA20ox1* allele, whereas both alleles were apparent for genotypes with high temperature response. We

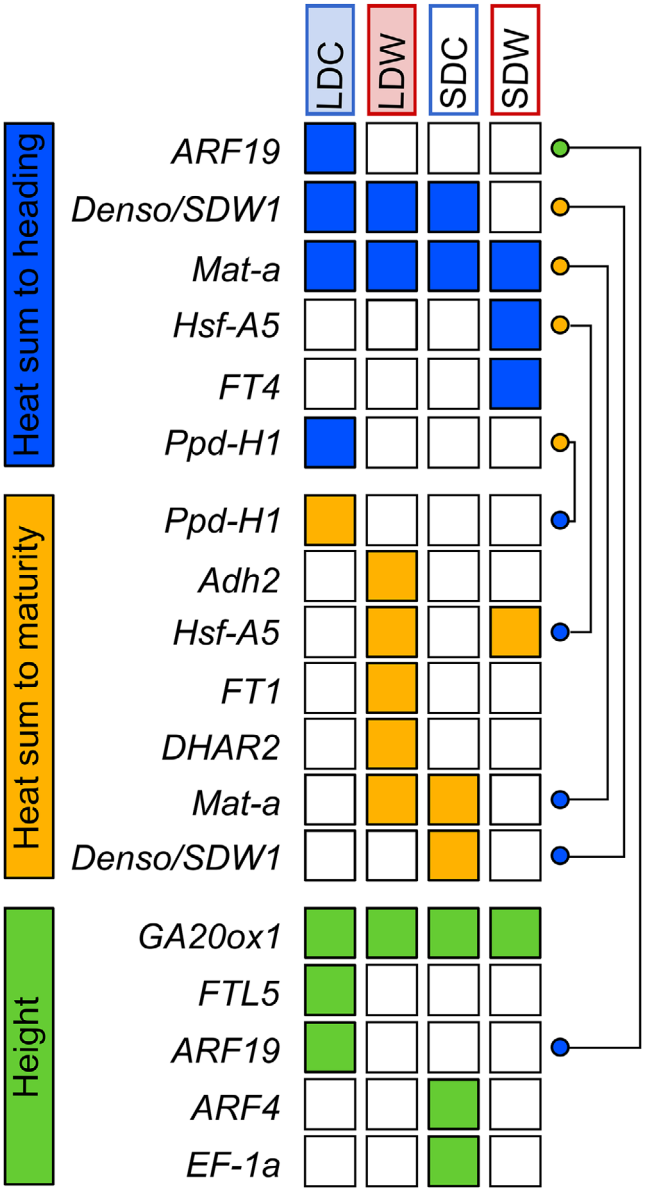

FIGURE 7 Overview of marker trait associations (MTAs) with putative loci and which of the four environments long day cold (LDC; 20-h day length and 15/5 ˚C, day/night), long day warm (LDW; 20-h day light and 25/15 ˚C), short day cold (SDC; 12-h day length and 15/10 ˚C), and short day warm (SDW; 12-h day length and 25/15 ˚C) they were found

speculate whether *GA20ox1* can compensate for the loss of function of *GA20ox2* in low temperature by downregulating GA production. However, as this was only seen in a subset of the genotypes with the same SNP for *GA20ox1*, more loci are presumably involved in this compensation. Interestingly, the genotypes with weakest height response, and hence highest height stability, to temperature were all selected in a cold environment. In particular, the temperature insensitive lines Teista II and Tampar qualify for further studies to reveal the genetics behind their height stability.

Two recent studies have performed GWAS on comparable panels of Nordic spring barley. Wonneberger et al. (2017) analyzed MTAs for heading and height in a set of Nordic spring barley lines grown under field conditions in Norway, and Göransson et al. (2019) performed GWAS for HSHD, HSMD, and height for another spring barley panel of Nordic origin grown under field conditions in the Nordic countries and Germany. The present study shared no identical MTAs with Wonneberger et al. (2017), whereas three MTAs were identical for both HSHD and HSMD, and one MTA for HSMD with the previous study by Göransson et al. (2019). The MTAs for both HSHD and HSMD were SCRI_RS_15171 (3H, *denso/sdw1*), SCRI_RS_164290 (3H, *denso/sdw1*), SCRI_RS_187343 (6H, *Hsf-A5*), and only for HSMD: SCRI_RS_193132 (3H, *denso/sdw1)*. The three markers on chromosome 3H were all localized near (<3.7 Mbp) the *denso/sdw1* locus. *Denso/sdw1* has previously been reported to influence height and heading (e.g. Jia et al., 2009). *Hsf-A5* is a drought and heat-stress regulator (Reddy et al., 2014) and interestingly, this MTA was only found in the warm environments where the highest temperatures were registered.

The accurate identification of MTAs for row type near the expected loci *Int-c* (0.3 Mbp) and *Vrs1* (0.9 Mbp) (Supplemental Table S6) provided confidence in the mapping results for other traits, even though the spring barley panel evaluated here is smaller than most comparable GWAS studies. The MTAs highlighted in the results were located within or near (<3.7 Mbp) previously known loci for the respective trait indicating that the peaks are true associations and not false positives. The PCA based on the genetic data showed a grouping according to row type. The day-length-neutral lines and the group with delayed heading in SDW did not form subgroups, indicating that this is rather an effect of few loci rather than an effect of the general genetic diversity. Heritability of the traits were similar or slightly higher than other recent studies (Göransson et al., 2019; Wonneberger et al., 2017), which found the broad-sense heritability for heading and height in field conditions to be in the range of 0.6–0.9. In controlled conditions like the current study, it is expected that the heritability is higher because of higher phenotyping precision. A controlled environment can never fully mimic field conditions. Nevertheless, a targeted selection in a northern environment has had impact on key traits. Icelandic barley lines showed a unique pattern of early maturity not found elsewhere and this can be partly assigned to an allelic difference in the *Ppd-H1* locus. Better understanding of the genetic mechanisms behind heading and maturity in low temperature in barley can work as a model for other crops when the production area is expanded northward. Such knowledge is essential for any breeding program where these are the main objectives, such as in Iceland. Looking past the present challenge when the tools for early maturity and height stability are available, the focus could be moved to combine these traits with more

quality traits and/or try to improve the yield of these cultivars. Many Icelandic lines lacked temperature induced stem elongation in low temperature, which was otherwise widely observed in the Nordic barleys studied. This is an advantage in the windy and cool Icelandic environment where the barley plants would otherwise be prone to lodging during the grain-filling period before harvest. This stresses the importance of selecting breeding lines in the target environment. The combination of *GA20ox1* and *denso/sdw1* (*GA20ox2*) has an impact of height control in low temperature, and furthermore the interaction of *Ga20ox1* with known earliness loci merits further studies. A validation of these results in field conditions with a larger panel would be necessary to fully conclude this pattern. Another way would be to construct a segregating population with diversity in the three *GA20ox* loci to further explore their effects on height and earliness in cold temperature.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Paper III

frontiers in Plant Science

ORIGINAL RESEARCH
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The Winter-Type Allele of *HvCEN* Is Associated With Earliness Without Severe Yield Penalty in Icelandic Spring Barley (*Hordeum vulgare* L.)

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Icelandic barley genotypes have shown extreme earliness both in flowering and maturity compared to other north European genotypes, whereas earliness is a key trait in adapting barley to northern latitudes. Four genes were partially re-sequenced, which are *Ppd-H1*, *HvCEN*, *HvELF3*, and *HvFT1,* to better understand the mechanisms underlying this observed earliness. These genes are all known to play a part in the photoperiod response. The objective of this study is to correlate allelic diversity with flowering time and yield data from Icelandic field trials. The resequencing identified two to three alleles at each locus which resulted in 12 haplotype combinations. One haplotype combination containing the winter-type allele of *Ppd-H1* correlated with extreme earliness, however, with a severe yield penalty. A winter-type allele in *HvCEN* in four genotypes correlated with earliness combined with high yield. Our results open the possibility of marker-assisted pyramiding as a rapid way to develop varieties with a shortened time from sowing to flowering under the extreme Icelandic growing conditions and possibly in other arctic or sub-arctic regions.

Keywords: barley, flowering time, yield, adaptation, *HvCEN*, *Ppd-H1*, *HvFT1*, *HvELF3*

INTRODUCTION

Barley has proven to be well-suited in harsh climates since its early domestication and is by far the most important cereal crop in Iceland and many other northern and arid regions of the world (Schoppach et al., 2017). Barley is thought to have moved gradually northwards from the center of early domestication and finally been introduced to Scandinavia around 2,000 BCE (Bogucki, 2000), approximately 2,000 years after the first farmers arrived in northern Europe (Skoglund et al., 2012). This lag might be explained in part by a lack of genetic variants suiting the special conditions in northern Europe, such as the shorter growing period and longer days. Sub-arctic agriculture is still at the margin of barley cultivation due to its characteristics such as short and cool growth period, occasional very strong winds, risk of frost in both late spring and early autumn, and a long photoperiod. This is highlighted by the relatively short history of barley cultivation in Iceland wherein barley has been continuously grown in the country since 1923 (Hermannsson, 1993). This is further accentuated by the high variation in yield and dry matter weight experienced by Icelandic farmers (Hilmarsson et al., 2017).

Despite the short history of barley cultivation in Iceland, it is being grown today at around 5,000 ha with an average yield of 3.2 t ha^{-1} (Hilmarsson et al., 2017), which is comparable to similar latitudes in Scandinavia. For example, the yield has been reported at 3.5 t ha^{-1} in the Trøndelag region in Norway (Lillemo et al., 2010). Several possible non-exclusive explanations for the increased barley cultivation in Iceland have been raised, which include favorable climate change, increased testing of Nordic breeding material leading to better selections of varieties, and a local breeding project aimed at breeding varieties especially adapted to the Icelandic climate (Hilmarsson et al., 2017). The Icelandic barley breeding program was initiated in 1990 and has mostly used Nordic material, focusing on adapting the highest yielding cultivars to local conditions by altering their flowering time and improving lodging and wind resistance. The study of Hilmarsson et al. (2017) recently described that the most promising breeding lines have been in field trials as a part of the breeding program together with foreign cultivars in four to five locations around the country every year.

The temperature during summer in Iceland is considerably lower than in other comparable latitudes. It has an average accumulated heat sum over the growth season of 1,300-day degrees (Ólafsson et al., 2007) compared to the Trøndelag region, which has an average heat sum of 1,790-day degrees (Hole and Rafoss, 2010). The study of Mølmann et al. (2021) reviewed how the uniquely long summer days in sub-arctic latitudes can compensate for below-optimal temperature during the growth season for biomass production in forage grasses. It seems that the phenological development of barley under these unique conditions is also different from that observed under higher temperatures (Göransson et al., 2021).

It is necessary to identify the specific alleles underlying traits to obtain the desired breeding targets with the least amount of breeding effort and to understand the interactions between different alleles and genes under different climatic conditions. Underlying traits include earliness, lodging resistance, straw strength, and frost tolerance.

Climate change is predicted to lead to increased temperatures, which will be more pronounced in the sub-arctic region than what is expected on average in the other regions globally (IPCC, 2018). With climate change coinciding with the need to increase global food production to keep up with the rising population, it is important to find ways to diversify the grassland-based agriculture commonly practiced in northern latitudes and to respond to increased droughts in the more arid parts of the world. Many of the globally important grain-producing regions are expected to suffer from severe drought and heat stress, which most likely will have a negative impact on the yield in these regions (Lobell et al., 2008). It is likely that other areas at comparable latitudes (and photoperiods) to Iceland will open as potential barley production regions in the near future based on predictions about future climate scenarios by IPCC (2018). The early flowering Icelandic breeding lines could be a valuable resource for developing high-yielding cultivars for wider distribution in northern latitudes, given that the early flowering traits can be efficiently introgressed into elite barley varieties more suitable to the expected higher average temperature. Under increased aridification, wild relatives of both wheat (*Triticum dicoccoides*) and barley (*Hordeum vulgare* ssp. *spontaneum*) have been shown to respond to climate change by displaying increased earliness (Nevo et al., 2012). This implies that even barley cultivation in more southern locations could benefit from increased understanding of earliness genes from Nordic breeding populations.

The transition from the vegetative state to flowering is controlled by a complex molecular system well-conserved between even distantly related plant species (Blümel et al., 2015; Hill and Li, 2016). Wherein the model organism *Arabidopsis thaliana* has served as an important reference for identification and verification of flowering-related genes (Andrés and Coupland, 2012). Several genes associated with the transition from vegetative growth to flowering in barley have been described and corresponding markers developed, e.g., Cockram et al. (2007), Faure et al. (2012), Varshney et al. (2007), and Wang et al. (2010). In the study of Zakhrabekova et al. (2012), they described a 4 bp deletion in the *HvELF3* gene (syn. *EAM8*, *mat-a*; orthologous to *ELF3* in *Arabidopsis thaliana*) in the two-row *cv.* "Mari" and suggested that this deletion might explain its earliness and day length neutrality. It also combined with its resistance to lodging allowing the extension of two-row barley into Northern Scandinavia and Iceland. This and similar studies enable the screening of breeding lines with allele-specific markers further opening the door to marker-assisted selection (MAS), a rapid method for introgressing specific trait-related alleles into selected lines (Muthu et al., 2020).

The effect of flowering genes on yield in barley has been reported from regions such as Jordan, where the day length sensitive allele of the *Ppd-H1* was associated with a 30% yield increase compared to the insensitive *ppd-H1* allele (Wiegmann et al., 2019). In northern Europe, the situation is reversed, and the non-responsive *ppd-H1* allele has been associated with increased yield (Wiegmann et al., 2019), highlighting the adaptive importance of *Ppd-H1*. *HvCEN* has been associated with yield and yield-related traits in studies in Australia where yield was positively associated with a QTL very near *HvCEN* (Obsa et al., 2017). *HvCEN* was the peak marker associated with yield in a spring-type × winter-type population tried in Mediterranean conditions, and they reported early heading without yield penalty (Tondelli et al., 2014). In all, 13 haplotypes of *HvCEN* have been described worldwide, out of which types I, II, and III are the most prevalent (Fernandez-Calleja et al., 2021). In the last exon, an SNP codes the amino acid Ala135 in types I and III whereas in type II it codes Pro135 (Comadran et al., 2012). The Ala135 coding haplotype is primarily found in European spring barley and is beneficial in long and cool growth seasons, where it delays flowering to allow for full utilization of the growth season. The Pro135 coding haplotype is beneficial in Mediterranean conditions where early flowering is beneficial to develop kernels before the summer heat (Comadran et al., 2012; Fernandez-Calleja et al., 2021). Sharma et al. (2018) found allelic diversity in *HvFT1* originating from wild barley, which had a reducing effect on yield. Similarly, Wiegmann et al. (2019) found the recessive wild "winter type" allele to reduce yield in a drought-stressed environment. HvELF3 has a defined role in the flowering

pathway by downregulating the HvFT1 gene (Boden et al., 2014) but its effect on yield-related characters is less defined.

Implementation of MAS in the breeding program will speed up the selection process, in particular for traits controlled by single or few genes, and add precision by enabling finesses that are difficult to control by traditional breeding such as pyramiding of multiple genes interacting on the same trait (Muthu et al., 2020). Establishing knowledge of available allelic diversity for example for earliness genes by applying allele-specific markers on the Icelandic and Scandinavian barley breeding material will provide the foundation for more precision in the future breeding program.

Two studies on the genetic mechanisms behind the Icelandic early barley have been published. The study of Göransson et al. (2019) identified allelic differences between single nucleotide polymorphism (SNP) markers at the *HvPph-H1*, and *HvFT1* loci as a partial explanation of the extreme earliness in the lines "247-1" and "247-11" observed in multi-location field trials ranging from Bavaria, Germany in the south to Iceland in the north. Göransson et al. (2021) identified variation at *HvELF3*, *Ppd-H1*, and *HvFT1* as contributing to early maturity in Icelandic barley, in an experiment with contrasting day length and temperature combinations in controlled environments. Comadran et al. (2012) highlighted the contribution of allelic variation at *HvCEN* as a factor to enable latitudinal range extension of barley and recently, whereas Bustos-Korts et al. (2019) and Jayakodi et al. (2020) have reported variation at the *HvCEN* locus as contributing to early flowering.

With this as a background, the objectives of the present study were to re-sequence the four earliness loci *Ppd-H1*, *HvFT1*, *HvELF3*, and *HvCEN* in a set of early Icelandic barley lines and their progenitors to elucidate the allelic variation behind the extreme earliness, which has been especially pronounced in lines "247-1" and "247-11".

MATERIALS AND METHODS

Icelandic Field Tests and Material Selected for Sequencing

Agronomic Data

Twenty barley genotypes (**Table 1**) were selected by analyzing data from geographically dispersed Icelandic cultivar trials between the years 1987 and 2014 (Hilmarsson et al., 2017). The selection was made to include (1) previously known extremely early lines ("247-11" and "247-1") (Göransson et al., 2019, 2021), (2) selected ancestors based on pedigree data, and (3) cultivars and breeding lines with a high yielding capacity in Iceland. The panel included Icelandic cultivars and breeding lines as well as foreign cultivars that had been tried in a minimum of four trials with yield data. The traits analyzed were yield (t ha^{-1}) and heading (number of days from sowing to heading). The heading was scored as the number of days from sowing until half of the plants in the plot had reached Zadoks growth stage 55 (Zadoks et al., 1974). This comparison included 1,608 data points for "yield" (ranging from four to 332 data points for different genotypes) and 814 data points for "heading" (ranging from two to 200 data points for different genotypes). Due to the highly unbalanced data set, best linear unbiased estimates (BLUEs) for heading and yield were calculated using the least square means (LS Means) model with package lme4 in R (Bates et al., 2015). The data were analyzed in a single stage (Piepho et al., 2012; Bernal-Vasquez et al., 2014) using genotype, location, and year as fixed effects whereas replication was a random effect nested within location × year. Based on the BLUEs for heading and yield, an index was calculated by dividing Yield with Heading (DDY) to estimate the lines with the highest yielding capacity and the shortest heading time. The significance between DDY was calculated with Tukey's test with grouping by haplotype combination using package dplyr in R (Wickham et al., 2021).

Resequencing Panel

Twenty additional genotypes were included for resequencing based on their role as parental lines to selected barley varieties or breeding lines of interest, which resulted in a panel of 40 genotypes (**Table 1**). The pedigree of the Icelandic breeding lines with their ancestors back to the relevant landraces, wherever possible, was compiled based on resources such as logbooks, information from breeding companies and gene banks, official cultivar registration data, and scientific publications, e.g., Manninen and Nissilä (1997) and Nurminiemi et al. (1996). This allowed the selection of the relevant ancestors for sequencing (**Supplementary Figure 1**). Among the 40 genotypes, 20 were two-rowed and 20 were six-rowed, 39 were characterized as spring barley, and the winter barley cultivar "Fimbul" was included based on pedigree data.

DNA Isolation and PCR Amplification

Deoxyribonucleic acid was isolated using the NucleoSpin® Plant II kit from Macherey-Nagel, Dueren, Germany (http://www.mn-net.com/) following the protocol of the manufacturer. PCR primers were designed based on sequences available for the "Morex" *HvELF3* (syn. *Eam8*, *Mat-a*; NCBI GenBank accession number JN180296), *Ppd-H1* (GenBank accession number AY943294), *HvCEN* (syn. *Eps2s*, *eam6;* GenBank accession number JX648182), and *HvFT1* (syn. *Vrn3;* GenBank accession number EU007831). The varieties selected were genotyped for the "Mari" deletion through sequencing of a 200 bp PCR fragment from exon 2, amplified using primers *HvELF3_delMariF1* and *HvELF3_delMariR1* (nucleotide sequences deposited with NCBI GenBank under accession numbers MZ286789-MZ286828). There is a total of 3,128 bp from the *Ppd-H1* gene, which were amplified and sequenced using seven primer pairs, covering exons 1–8 (nucleotide sequences deposited with NCBI GenBank under accession numbers MZ286829-MZ286868). From the *HvCEN* gene, 1,001 bp were amplified and sequenced using three primer pairs (nucleotide sequences deposited with NCBI GenBank under accession numbers MZ286869-MZ286908), and 2,595 bp were amplified and sequenced using six primer pairs from the *HvFT1* gene (nucleotide sequences deposited with NCBI GenBank under accession numbers MZ286909-MZ286948). Same primers were used for PCR and sequencing, all listed in **Table 2** with information on sequence and annealing temperature.

TABLE 1 | Genotypes with row type information, and their haplotypes of four re-sequenced flowering genes: *HvELF3* (blue), *Ppd-H1* (orange), *HvCEN* (green), and *HvFT1* (grey), the geographic origin, release year, and note why they were included in the study.

	Genotype [row type]	*HvELF3*	*Ppd-H1*	*HvCEN*	*HvFT1*	Origin	Released	Note
H01	**250-4** [6r]	E2	P2	C2	F2	Iceland	Breeding line	High yield
	Arve [6r]	E2	P2	C2	F2	Norway	1990	In pedigree
	Åsa [6r]	E2	P2	C2	F2	Sweden	1942	In pedigree
	Asplund [6r]	E2	P2	C2	F2	Sweden	1910	In pedigree
	Maskin [6r]	E2	P2	C2	F2	Norway	1918	In pedigree
	Scots Bere [6r]	E2	P2	C2	F2	Scotland	Landrace	
	Sigur-F [6r]	E2	P2	C2	F2	Faroe Isl.	Landrace	In pedigree
	Tammi [6r]	E2	P2	C2	F2	Finland	1937	In pedigree
	Tampa [6r]	E2	P2	C2	F2	Faroe Isl.	Landrace	In pedigree
	Varde [6r]	E2	P2	C2	F2	Norway	1941	In pedigree
	Vigdis [6r]	E2	P2	C2	F2	Norway	1964	In pedigree
	VoH2825 [6r]	E2	P2	C2	F2	Norway	Breeding line	In pedigree
H02	**263-9** [2r]	E2	P2	C2	F3	Iceland	Breeding line	High yield
	Binder [2r]	E2	P2	C2	F3	Denmark	1913	In pedigree
	Gull [2r]	E2	P2	C2	F3	Sweden	1913	In pedigree
	Kannas [2r]	E2	P2	C2	F3	Sweden	2013	High yield
	Kria [2r]	E2	P2	C2	F3	Iceland	2005	In pedigree
	Monte-Christo [6r]	E2	P2	C2	F3	India	Landrace	In pedigree
	Skegla [2r]	E2	P2	C2	F3	Iceland	2002	In pedigree
H03	Akka [2r]	E1	P2	C2	F1	Sweden	1970	
	Arla [2r]	E1	P2	C2	F1	Sweden	1962	
	Deba abed [2r]	E1	P2	C2	F1	Denmark	1964	In pedigree
	Minttu [2r]	E1	P2	C2	F1	Finland	2005	High yield
	Triumph [2r]	E1	P2	C2	F1	Germany	1973	In pedigree
H04	**Is-046** [2r]	E3	P2	C2	F1	Iceland	Breeding line	In pedigree - DLN
	Mari [2r]	E3	P2	C2	F1	Sweden	1960	In pedigree - DLN
	Teista [2r]	E3	P2	C2	F1	Iceland	2021	In pedigree - DLN
H05	Opal [2r]	E2	P2	C2	F1	Denmark	1922	In pedigree
	Pernilla [2r]	E2	P2	C2	F1	Sweden	1979	In pedigree
	Swallow [2r]	E2	P2	C2	F1	Germany	1962	In pedigree
H06	**06-130** [6r]	E2	P2	C1	F1	Iceland	Breeding line	High yield
	292-51 [6r]	E2	P2	C1	F1	Iceland	Breeding line	High yield
H07	**292-2** [6r]	E2	P2	C1	F2	Iceland	Breeding line	High yield
	Wolmari [6r]	E2	P2	C1	F2	Finland	2010	In pedigree
H08	**247-1** [6r]	E2	P1	C2	F2	Iceland	Breeding line	Extreme Earliness
	247-11 [6r]	E2	P1	C2	F2	Iceland	Breeding line	Extreme Earliness
H09	Fimbul [6r]	E1	P1	C1	F1*	Sweden	1946	In pedigree
H10	Golf [2r]	E1	P3	C2	F1	UK	1983	In pedigree
H11	**Nairn** [2r]	E2	P1	C2	F1	Scotland	1984	In pedigree
H12	**Saana** [2r]	E3	P2	C2	F2	Finland	1996	High yield

*The 20 genotypes marked in bold indicate that heading day and yield data have been analyzed (see **Figure 1**). H01–H12 indicate haplotype combinations. DLN indicates day-length neutral lines identified in Göransson et al. (2021).*

PCR products were purified using a NucleoSpin® PCR clean-up Gel extraction kit from Macherey-Nagel according to the recommendations of the manufacturer.

Bioinformatics Analysis

Sequencing results obtained were aligned to the relevant reference sequence and consensus sequences created for each gene for every variety sequenced using Geneious R11 (www.geneious.com). Open reading frames (ORFs) were translated using Geneious R11 and saved as a separate file. The resulting variety-specific consensus sequences, both nucleotide sequences and amino acid sequences, were compared to sequences publicly available in NCBI GenBank using multiple align features in Geneious R11 and haplotypes constructed manually. To identify potential significant effects on yield and heading for each of the four loci, BLUEs were compared using one-way analysis of variance (ANOVA) in Minitab® 19 (version 19.2020.1, Minitab LLC, State College, Pennsylvania, USA).

TABLE 2 | Primer names and sequences used for re-sequencing four flowering genes, along with their annealing temperature (Tm).

Primer name	Gene	Sequence (5′-3′)	Tm
Hvul_PpdH1-F1	Ppd-H1	5′-CGACTGTCATTCACGGCC-3′	58.5
Hvul_PpdH1-F2	Ppd-H1	5′-TGCTGTTGCTGCTGGCTC-3′	58.2
Hvul_PpdH1-F3	Ppd-H1	5′-TTGTTTGGACTTTGGATAAACTTG-3′	55.9
Hvul_PpdH1-F4	Ppd-H1	5′-AGAATACTTACATGTGTGAGAAGT-3′	55.9
Hvul_PpdH1-F5	Ppd-H1	5′-GCAAAGCATAATATCAGTGTCCT-3′	57.1
Hvul_PpdH1-R1	Ppd-H1	5′-GTAGCAGTATACCTTAAGTACA-3′	54.7
Hvul_PpdH1-R2	Ppd-H1	5′-AGCTTCCTTATCCTAACAATTGT-3′	55.3
Hvul_PpdH1-R3	Ppd-H1	5′-ACGGATGATTTCAGGATTCAC-3′	55.9
Hvul_PpdH1-R4	Ppd-H1	5′-GTACTAGGTATAGCTAGGTGCG-3′	60.3
Hvul_PpdH1-R5	Ppd-H1	5′-ACAAGAATCAGCTGTCTAATTAGT-3′	55.9
Hvul_PpdH1-F6	Ppd-H1	5′-TCCAACCCCACTCGCCG-3′	60.0
Hvul_PpdH1-R6	Ppd-H1	5′-ACAAGATAAGTATTGGTGGAGC-3′	56.5
Hvul_PpdH1-F7	Ppd-H1	5′-CTCAAGTGCCCAACCAGC-3′	58.2
Hvul_PpdH1-R7	Ppd-H1	5′-GGAACTTAATCAATACGAAGTGG-3′	57.1
Hvul_PpdH1-F8	Ppd-H1	5′-CCAGTGTTGTCAATCCTTCGG-3′	59.8
Hvul_PpdH1-R8	Ppd-H1	5′-CTGAATGAGTTGCTACCATAGTTGG-3′	61.3
Hvul-PpdHl-R9	Ppd-H1	5′-GAGACGCGGAATTTTATTTAAC-3′	54.7
Hvul_PpdH1-R1b	Ppd-H1	5′-TGTCTGAAAATATTACAGGTAGC-3′	55.3
Hvul_PpdH1-F9	Ppd-H1	5′-ATAATGGCAGTGGCACTC-3′	53.7
Hvul_PpdH1-R9b	Ppd-H1	5′-GACTGATCCGGAGACATG-3′	55.9
Hvul_PpdH1-F10	Ppd-H1	5′-TTGTCAATCCTTCGGGTC-3′	53.7
Hvul_PpdH1-R10	Ppd-H1	5′-CTTCTTCCAGGAGATGAGAC-3′	57.3
HvCEN_F1	HvCEN	5′-AACTTTTCAGTTCAAGCTAGG-3′	54.0
HvCEN_F2	HvCEN	5′-ATCCATACCTGAGGGAGCACC-3′	61.8
HvCEN_F3	HvCEN	5′-TCCTTTTCATGCATGACTTGC-3′	55.9
HvCEN_R1	HvCEN	5′-TAAACTAGCTTGGGTTAGTGG-3′	55.9
HvCEN_R2	HvCEN	5′-AAATTAAGGATGGGGCCAATC-3′	55.9
HvCEN_R3	HvCEN	5′-AAGAGAAGAAGGGTATGGCTG-3′	57.9
HvELF3_delMariF1	HvELF3	5′-CTTCATCGTTTCAATTTTCTGCAG-3′	57.6
HvELF3_delMariF2	HvELF3	5′-ATTTTCTGCAGACAAGACAATGGG-3′	59.3
HvELF3_delMariR1	HvELF3	5′-CTGCACATCGTGATGTCCGGTTGA-3′	64.4
HvELF3_delMariR2	HvELF3	5′-CATCCCTCTGCACATCGTGATGTC-3′	64.4
Hvul_FT-R3	HvFT1	5′-GAAGGGGTCCAGCACG-3′	56.9
Hvul_FT-F3	HvFT1	5′-GATCCATCCATCGGTCTC-3′	56.0
Hvul_FT-F4	HvFT1	5′-ATCCGCTGGTTGTCGG-3′	54.3
Hvul_FT-F1	HvFT1	5′-GAATGTATCTACGATCAAGG-3′	53.2
Hvul_FT-F5	HvFT1	5′-TGATCATGATATGTGCATGC-3′	53.2
Hvul_FT-R5	HvFT1	5′-TAATGCTTAATTCGTGGCTGG-3′	55.9
Hvul_FT-R1	HvFT1	5′-CTGCGAGAATATATAAATGCC-3′	54.0
Hvul_FT-F2	HvFT1	5′-TGGAGCATCATTTCGTCC-3′	53.7
Hvul_FT-R4	HvFT1	5′-TAGGACTTGGAGCATCTGG-3′	56.7
Hvul_FT-R2	HvFT1	5′-TGGTTCAATCGCCAGAGC-3′	56.0
Hvul_FT-F2a	HvFT1	5′-TTATTTCAGCCCAGGGAC-3′	53.7
Hvul_FT-R2a	HvFT1	5′-TGTGGACGTGGTTCAATC-3′	53.7

RESULTS

Phenotypic Differences and Sequencing Results

The best linear unbiased estimates for yield ranged from 1.61 to 4.43 t ha^{-1} and for heading from 66 to 83 days from sowing to heading (**Figure 1**). Three Icelandic breeding lines were extremely early with a heading day <70 days in the cold Icelandic climate. Two of them ("247-11" and "247-1") were low yielding (1.61 and 2.28 t ha^{-1}, respectively), whereas the third ("06-130") yielded above average at 3.66 t ha^{-1}. The DDY index ranged from 0.024 – 0.058 for the Icelandic breeding lines "247-11" and "250-4", respectively (**Figure 2**). Despite the limited number of observations, there was a significant difference between the four genotypes with the highest DDY and the two genotypes with the lowest, respectively ($p = 0.03$).

Resequencing of the four genes, *HvELF3*, *Ppd-H1*, *HvCEN*, and *HvFT1* resulted in two to three allelic variants per gene with 12 haplotype combinations in the panel of Icelandic and north European genotypes (**Table 1**). Resequencing of the *HvELF3* gene identified two polymorphic sites giving rise to three haplotypes, referred to here as *HvELF3*^{E1}, *HvELF3*^{E2}, and *HvELF3*^{E3}. The E2 allele is a single nucleotide polymorphism changing C in the reference sequence to a G and leading to a Glycine 316 to Alanine substitution (p.G316A), with the E3 allele including a 4 bp deletion in exon 2 (the so-called "Mari deletion") in addition to the G to C SNP (**Supplementary Figure 2**). Among

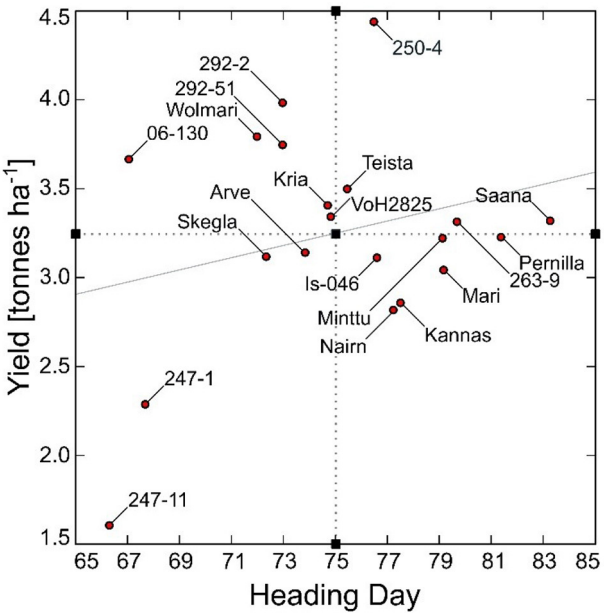

FIGURE 1 | Yield (t ha^{-1}) and heading day (days from sowing to heading) for 20 barley genotypes under Icelandic environmental conditions for the period 1987–2014. The mean values for heading day and yield are indicated as black boxes on the x- and y-axes, respectively, and the trendline is indicated as the gray slope.

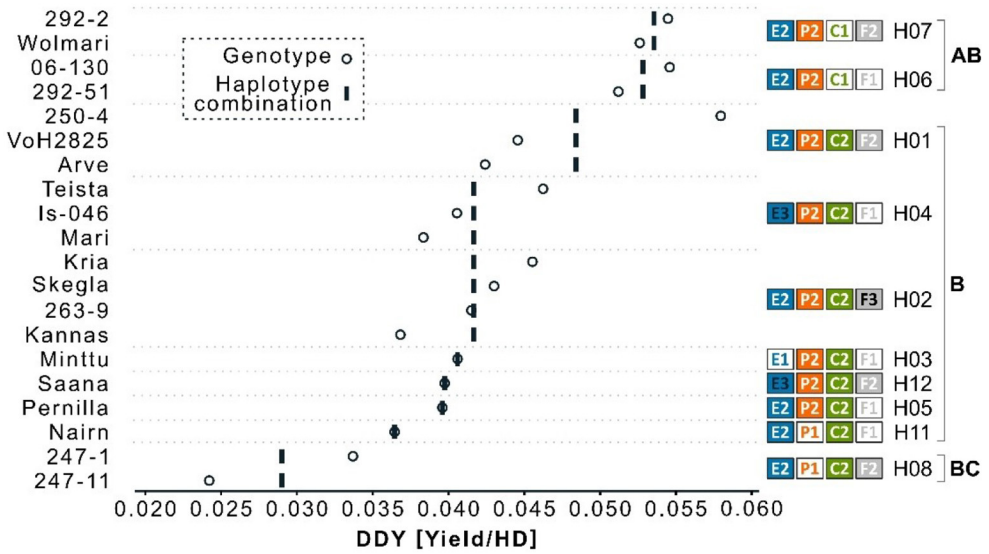

FIGURE 2 | Index values of yield by heading day (DDY) for 20 barley genotypes. Open circles indicated DDY for each genotype. Vertical bands indicate the mean per haplotype combination. Haplotype combinations for the four flowering loci *HvELF3* (blue, E), *Ppd-H1* (orange, P), *HvCEN* (green, C), and *HvFT1* (gray, F) are shown to the right of the diagram. Tukey's test for significance resulted in a significant difference between haplotype H08 and H07/H06 ($p = 0.03$) and is indicated with the letters to the far right.

the 40 genotypes sequenced, 29 carried the allele $HvELF3^{E2}$ and four lines carried the deletion allele found in the variety "Mari" (**Table 1**). There was no significant allele effect in *HvELF3* for either heading day ($p = 0.11$) or yield ($p = 0.999$) (**Supplementary Tables 1**, **2**).

Sequencing of the *Ppd-H1* gene in our panel identified 16 polymorphic sites, including 13 polymorphic sites in the coding regions of the gene, with the remaining polymorphisms in intronic regions (**Figure 3**). These polymorphisms lead to three haplotypes, referred to here as $Ppd\text{-}H1^{P1}$, $Ppd\text{-}H1^{P2}$, and $Ppd\text{-}H1^{P3}$, with the P2 haplotype being the most frequent one which is found in 35 of the 40 lines sequenced. The $Ppd\text{-}H1^{P2}$ allele is the previously reported mutated allele with seven missense mutations, which results in reduced photoperiod sensitivity commonly found in spring barley (Turner et al., 2005). Haplotypes P2 and P3 are only slightly different from each other with just a difference of two polymorphic sites, one found in intron 1 and the other being a missense polymorphism in exon 1 (p.Q17H) (**Figure 3**), with haplotype P3 only found in the variety "Golf" (**Table 1**). The $Ppd\text{-}H1^{P1}$ allele described here corresponds to the wild type *Ppd-H1* allele, found primarily in winter barley and regulating flowering in response to increasing day length (Turner et al., 2005). There was a significant allele effect on yield ($p = 0.00$) between the two alleles P1 and P2 of *Ppd-H1* where yield data was available but no significant difference in heading day ($p = 0.055$) (**Supplementary Tables 1**, **2**).

Sequencing of the *HvCEN* gene identified three polymorphic sites in our collection, two additional polymorphisms in comparison to *cv.* Bonus the reference sequence. One of which is found in coding regions of the gene leading to an amino acid change (p.A135P), with three additional polymorphic sites found in intron 2 (**Figure 3**). These polymorphisms give rise to two haplotypes referred to here as $HvCEN^{C1}$ and $HvCEN^{C2}$, with the C2 haplotype being by far the most common haplotype, which is found in 35 of the 40 lines sequenced (**Table 1**). The C1 haplotype was found in the winter barley cultivar "Fimbul" and four other spring barley genotypes (**Table 1**), all of which combined earliness with high yield (**Figure 4**). There was a significant difference between the three alleles of *HvCEN* for yield ($p = 0.036$) but not for heading day ($p = 0.064$) (**Supplementary Tables 1**, **2**).

Resequencing of the *HvFT1* gene resulted in 37 polymorphic sites, all of which are located outside the coding regions of the gene, with the vast majority found upstream of the first coding sequence (**Figure 3**). This leads to three different haplotypes, $HvFT1^{F1}$, $HvFT1^{F2}$, and $HvFT1^{F3}$, with considerable overlap between haplotypes F2 and F3, and almost equal frequencies of haplotypes in our panel of lines (**Table 1**). There was no significant allele effect in *HvFT1* for either heading day ($p = 0.466$) or yield ($p = 0.954$) (**Supplementary Tables 1**, **2**).

Effect of Haplotypes on Yield and Heading

Intersection analysis of haplotypes and barley lines identified 12 haplotype combinations of the 4 genes with the $HvELF3^{E2}/Ppd\text{-}H1^{P2}/HvCEN^{C2}/HvFT1^{F2}$ (E2/P2/C2/F2) combination being the most frequent, found in 12 of the 40 lines sequenced, with frequencies of other haplotype combinations ranging from one to seven (**Table 1**). The two extremely early Icelandic lines sequenced, which are "247-1" and "247-11", had the same haplotype composition, $HvELF3^{E2}/Ppd\text{-}H1^{P1}/HvCEN^{C2}/HvFT1^{F2}$ (see haplotype combination eight in

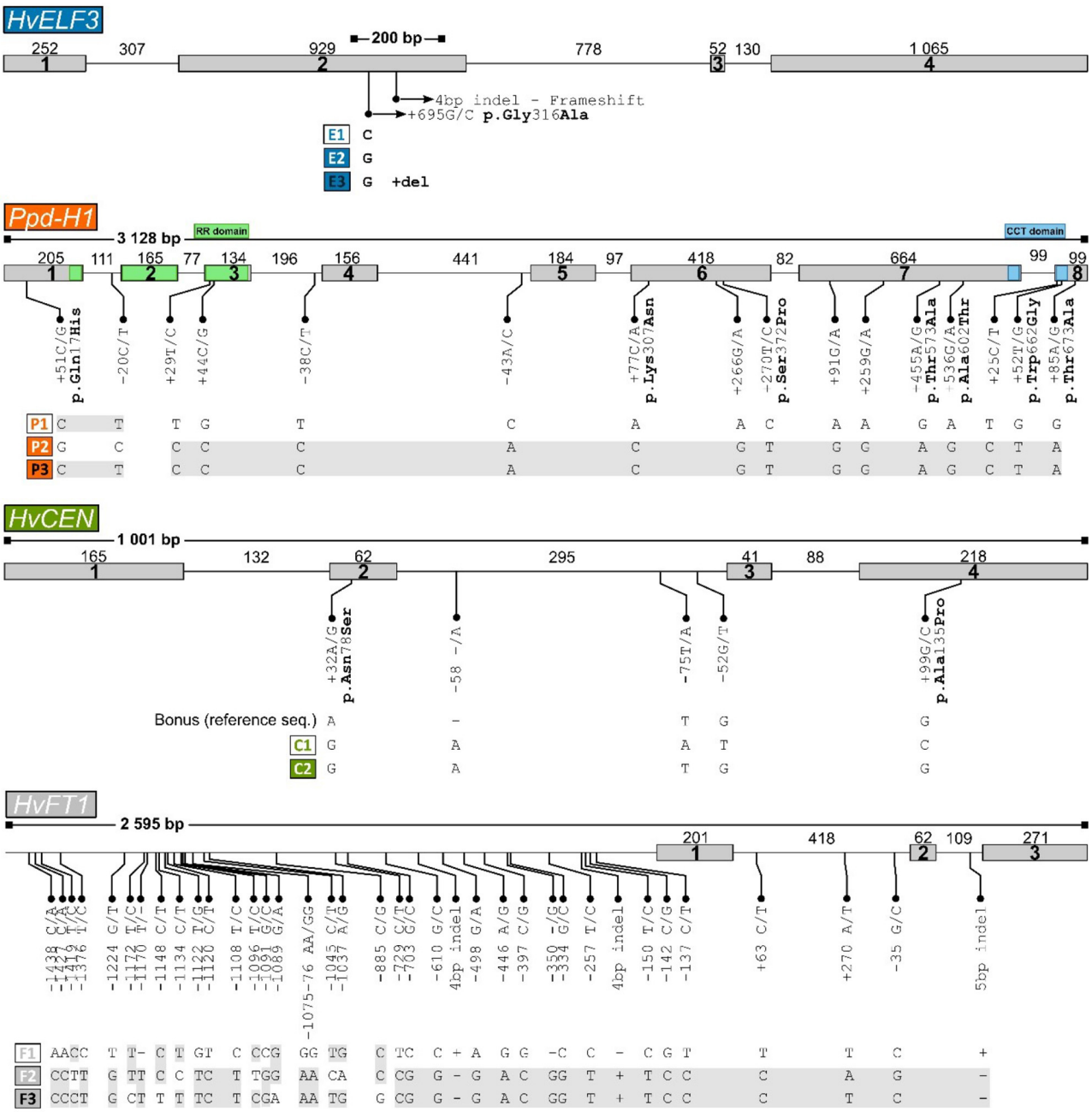

FIGURE 3 | Structure of the four genes re-sequenced, *HvELF3*, *Ppd-H1*, *HvCEN*, and *HvFT1*, with polymorphisms constituting haplotypes identified. Exons are shown as boxes (size in base pairs below) and introns as lines (size in base-pairs above). The size of the region sequenced is shown in base pairs above the intron-exon structure with lines with filled boxes at each end. For each gene, the identified haplotypes are indicated below the corresponding line drawings. In the structure of the *HvELF3* (*Mat-a*) gene the location of the 4 bp "Mari" deletion is indicated with an arrow. Color coding of genes is the same in all figures.

Figure 2). The four early lines with high yielding capacity had the haplotype composition $HvELF3^{E2}/Ppd\text{-}H1^{P2}/HvCEN^{C1}$ (**Figure 2**) differing only in *HvFT1*, with $HvFT1^{F1}$ found in lines "06-130" and "292-51" and $HvFT1^{F2}$ found in lines "292-2" and "Wolmari". The $Pph\text{-}H1^{P1}$ interacted with the $HvFT1^{F2}$ allele, where the combination P1F2 had reduced yield (**Supplementary Figure 3**).

Origin of Haplotypes in the Pedigree of Icelandic Lines

The pedigree of the Icelandic lines was reconstructed to understand the origin of the haplotype combinations found in the extremely early Icelandic lines, which are the low yielding sister lines "247-1" and "247-11" and the high yielding lines "06-130", "292-2" and "292-51" (**Figure 5**;

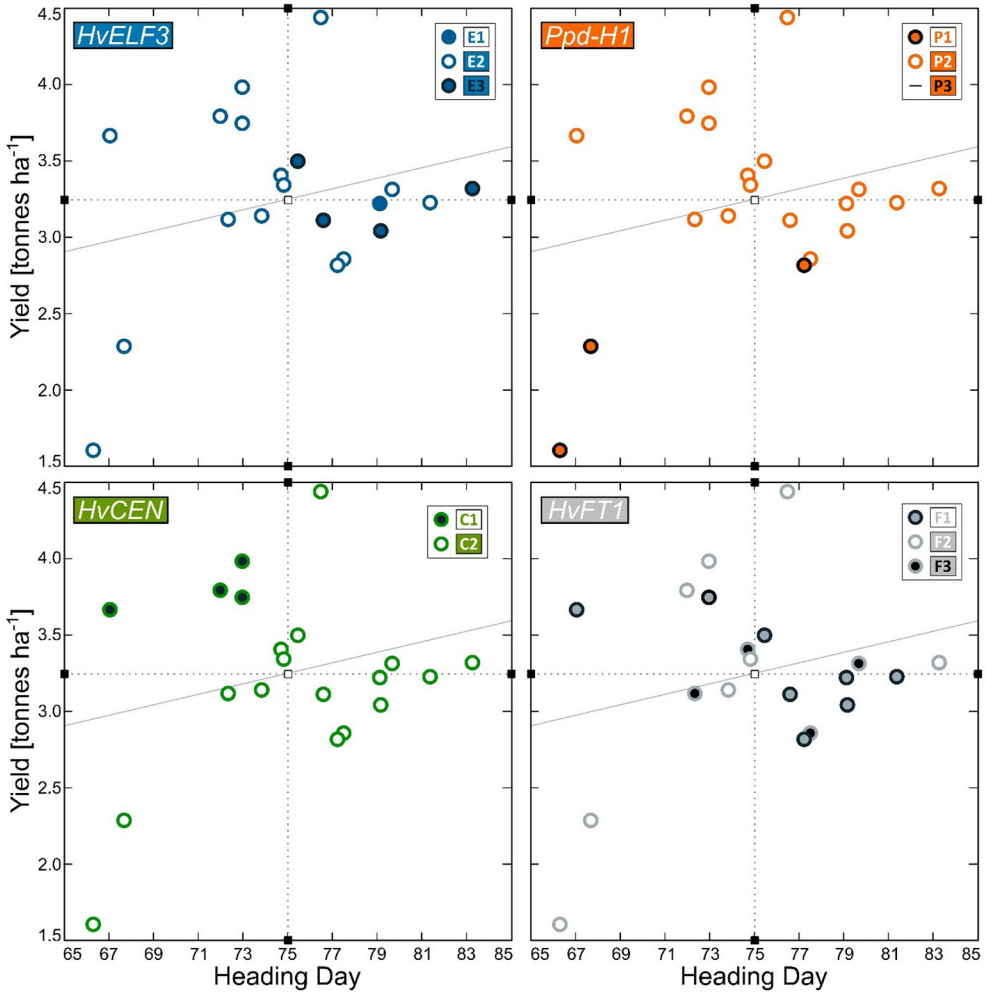

FIGURE 4 | Heading day (days from sowing to heading) and Yield (t ha^{-1}) for 20 barley genotypes grown in field trials in Iceland in the period 1987–2014. The four plots show results for the four re-sequenced genes *HvELF3* (blue), *Ppd-H1* (orange), *HvCEN* (green), and *HvFT1* (gray). The alleles are indicated with differently color-coded dots. Mean heading day and yield are indicated as dotted lines and the sloping gray line indicates the trendline.

Supplementary Figure 1). This analysis shows that all the earliest genotypes are related to "Arve" but less related on the other side of the pedigree. While the *HvELF3*^{E2}/*Ppd-H1*^{P2}/*HvCEN*^{C2}/*HvFT1*^{F2} haplotype combination is common in the pedigree for the two early Icelandic lines (see "Arve", "VoH2825", and "Vigdis" in **Figure 5**), the haplotype combination with *Ppd-H1*^{P1} (E2/P1/C2/F2 in **Figure 5**) is rare and is created with the haplotype inherited from "Nairn".

The four early- and high-yielding lines were of a less certain origin. "Wolmari" is a cultivar from Finland; "06-130" is an Icelandic breeding line (a cross between "Skumur I" and one of the Norwegian cultivars "Arve", "Olsok", "Lavrans", or "Tiril"); the sister lines "292-2" and "292-51", are crosses between the combined genotype of "Arve" × "SjD918011" and "Olsok" (**Figure 5**).

DISCUSSION

Controlling the period from sowing to heading is important to maximize the utilization of the growing period and therefore has a direct effect on yield. Early heading has been correlated with early maturity (Göransson et al., 2019) and also acts to minimize the negative effect of adverse events at the end of the growing season, whether it be drought, heavy rains, frost, or extreme winds. The Icelandic barley breeding project, e.g., Hermannsson (1993) and Hilmarsson et al. (2017), has resulted in cultivars and breeding lines of interest due to increased earliness that could help us to better understand the control of flowering time in barley.

The main finding is the effect of the *HvCEN*^{C1} allele on DDY showing that earliness and high yield are possible to combine in a northern environment, which opens up possibilities

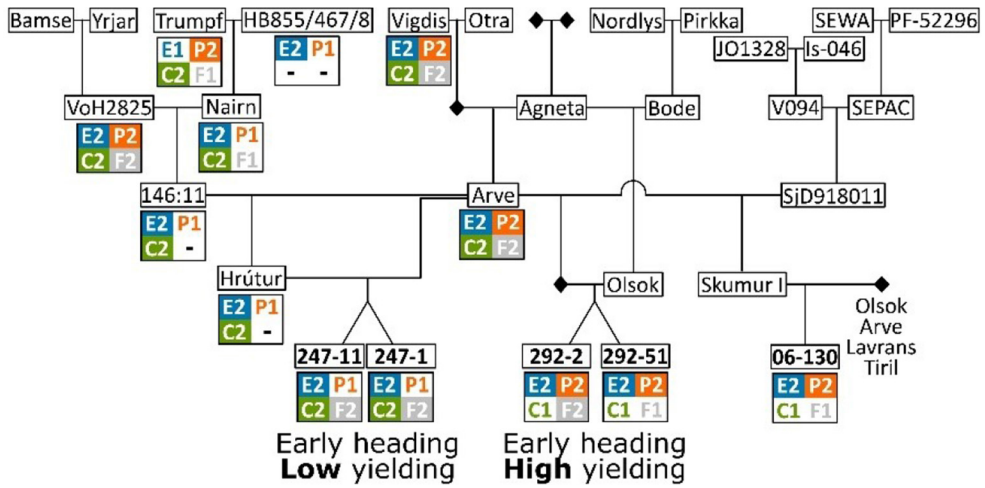

FIGURE 5 | Pedigree of five Icelandic barley breeding lines: "247-1", "247-11", "292-2", "292-51", and "06-130". Haplotypes for the four different genes re-sequenced are shown below each genotype. When possible, haplotypes are imputed for unsequenced genotypes. The full pedigree can be found in **Supplementary Figure 1**.

to breed for earlier barley cultivars without losing yield. The spring type allele of *HvCEN*^{C2} has previously been reported to be virtually fixed in northern European spring barley (Tondelli et al., 2013; Fjellheim et al., 2014) and in earlier studies by Genome-Wide Association Studies (GWAS) of Nordic material, *HvCEN* has remained undetected, presumably due to a low allele frequency of alternate alleles in the studied populations (Göransson et al., 2019, 2021). The study of Fernandez-Calleja et al. (2021) reviewed the effect of diversity in *HvCEN* within three alleles, which are I, II, and III, whereof numbers II and III are of interest for this study. The type II (from "Nure"), corresponding to *HvCEN*^{C1}, is predominantly found in winter barley and was in their study found to contribute to earliness and yield in the "Nure" × "Tremois" mapping population. The type III allele (from "Tremois") is predominantly found in spring barley cultivars and is corresponding to *HvCEN*^{C2} in this study. Furthermore, the review by Fernandez-Calleja et al. (2021) reported latitudinal distribution of the two alleles in European barley cultivars indicating an adaptive role of the type III allele (*HvCEN*^{C2}) in northern latitudes, originally reported in the studies of Comadran et al. (2012) and Bustos-Korts et al. (2019). In this study, we documented diversity in *HvCEN* in a panel of northerly adapted spring barley genotypes: three Icelandic genotypes and the Finnish spring barley cultivar "Wolmari" all carried the *HvCEN*^{C1} allele, which they also shared with the only winter barley included in the study, "Fimbul". Moreover, all four spring barley genotypes carrying the winter-type *HvCEN*^{C1} combined earliness with a yielding capacity above average. This highlights its potential contribution to combine the two traits earliness and yield. There have been ambiguous reports on the role of *HvCEN* in yielding capacity as reviewed by the study of Fernandez-Calleja et al. (2021). The type III allele has the effect to delay flowering and has been advantageous in the cold and long growth seasons of northern Europe. The type II allele induces early flowering and is advantageous to the onset of flowering immediately after the south European winter months to enable the plant to develop seeds before the onset of the warm and dry summer. It is speculated in this study whether the type II allele can have an advantageous effect in the extreme environmental conditions of Iceland, where a delayed onset of flowering by type III allele may mean that the grain filling period will not coincide with the highest mean summer temperature peak at 11°C in July and August (Icelandic Meteorological Office, 2021). This would enable plants carrying the type II allele to initiate flowering early enough to make use of the highest summer temperatures for energy allocation to the kernels.

The four lines with significantly higher DDY (**Figure 2**) differed in *HvFT1*, a difference which we could not explain from the data in this study. The results are inconclusive for the role of *HvFT1* in both earliness and yield. In the study of Casas et al. (2011), they identified variation in the promoter and first intron of the *HvFT1* gene in a panel of Spanish landraces. The study conducted by Nitcher et al. (2013) reported an increase in copy number variation (CNV) at the *HvFT1* locus to be associated with early flowering in barley. They reported the CNV to originate from the Finnish spring barley variety "Tammi" ("Olli"/"Asplund"). Loscos et al. (2014) reported CNV of *HvFT1* in Nordic barleys, including Asplund, Maskin, and Tammi, carried a specific combination of only one promoter and several copies of exon 1. These lines all carry *HvFT1*^{F2} in this study and should reportedly be the earliest allele (Loscos et al., 2014). Although it cannot be concluded on CNV in *HvFT1* from this resequencing study, this is still an indication that the *HvFT1*^{F2} allele is contributing to the early flowering as was previously described by the CNV reported by the study of Nitcher et al. (2013). The study of Fernandez-Calleja et al. (2021) proposed a nomenclature of the *HvFT1* alleles based on an AG/TC SNP polymorphism in the first intron, which would group the F2 allele of this study as *VRN-H3a(T),* and the F1 and F3 alleles would jointly be grouped as *vrn-H3c/d(n)*. More research is needed to

elucidate the role of *HvFT1* in the genetic background of early Icelandic spring barley.

The two Icelandic lines"247-1" and "247-11" have shown special earliness in earlier studies (Göransson et al., 2019, 2021) and we show in this study that this is most likely due to a unique combination of haplotypes at four loci all known to play a role in flowering. This unique haplotype combination was created by crossing the Norwegian cultivar "Arve" with an Icelandic breeding line that was a cross between "Hrutur" × "Arve", with the *Ppd-H1*^{P1} allele coming from "Nairn", which is a Scottish variety. This variety seems to have inherited that haplotype from the variety "Fimbul", a Swedish winter barley variety (**Figure 5**; **Supplementary Figure 1**). The winter-type allele *Ppd-H1*^{P1} is the wild-type and has been reported to advance flowering when day length increases. In nature, wild barley grows vegetatively during the wet winter months in the fertile crescent and is triggered by the increased day length in spring to initiate flowering before the summer heat and drought starts (Nevo et al., 2012). Most spring barley cultivars benefit from a delayed flowering in spring to establish vegetatively before transitioning to the reproductive stage, with the delay caused by the *Ppd-H1*^{P2} allele (Turner et al., 2005). The effect of the spring-type allele *Ppd-H1*^{P2} is well-studied in northern European barley, e.g., Turner et al. (2005) and Jones et al. (2008). We can now show that the winter-type contributes to extreme earliness among Icelandic spring barley genotypes. For the extremely early Icelandic barley lines we speculate that the *HvFT1*^{F2} from "Tammi" combined with the *Ppd-H1*^{P1} winter-type allele could be the reason for the extreme earliness of "247-11" and "247-1". However, this comes with a severe yield penalty. The yielding capacity of the genotypes carrying the winter-type *Ppd-H1*^{P1} allele is well below average indicating a negative tradeoff between *Ppd-H1* ^{P1} derived earliness and yield. There is a noteworthy interaction between *Ppd-H1*^{P1} and *HvFT1*^{F2} with reduced yield indicating an interaction between the two genes (**Supplementary Figure 5**). The Scottish variety of barley "Nairn", which carries the winter-type *Ppd-H1* ^{P1} allele without any effect on earliness or marked negative effect on yield, could here be benefitting from the haplotype combination of *Ppd-H1* ^{P1} and *HvFT1*^{F1}. This combination was unique for "Nairn" and indicates an epistatic effect between the two genes (**Supplementary Figure 5**), with the P1F1 combination has little effect on yield, whereas the yield is reduced in the P1F2 combination (present in the genotypes "247-11" and "247-1").

Allelic diversity at the *HvELF3* locus was less conclusive. *HvELF3* has previously been reported as the primary cause of adaptation to northern latitudes (Zakhrabekova et al., 2012). It can be concluded that the extreme earliness observed in "247-11", "247-1", and "06-130" is not caused by the Mari-deletion *HvELF*^{E3}, which causes day length neutrality. It should be noted that the sequenced region is a region around exon 2, which leaves other previously described diverse regions such as exon 4 (Xia et al., 2017) unexplored.

The haplotypes with the highest and lowest DDY (**Figure 2**) were segregated in the two loci *Ppd-H1* and *HvCEN*. Genotypes carrying the day length sensitive allele *Ppd-H1*^{P1} in combination with *HvCEN*^{C2} were early heading, but low yielding. Genotypes carrying the *HvCEN*^{C1} allele combined with *Ppd-H1*^{P2} were both early heading and high yielding. This study speculates whether there is an interaction between the two loci, but the study did not include any *Ppd-H1*^{P1}–*HvCEN*^{C1} haplotype, which would merit further studies. The allele *HvFT1*^{F2} seems to act epistatically to induce extreme earliness with winter-type alleles of both *Ppd-H1*^{P1} and *HvCEN*^{C1}. In this study, we could not observe any haplotype combination of *HvFT1*^{F2}/*Ppd-H1*^{P1}/*HvCEN*^{C1}. The number of identified alleles in the four loci gives a hypothetical number of 54 different haplotype combinations ($3 \times 3 \times 2 \times 3 = 54$), whereas in the current panel we found 12. The small number of genotypes (20) with phenotype data was a limitation in the statistic analysis and data interpretation. In addition, we can conclude that there exist more known polymorphic sites for all genes than was found here (**Supplementary Figure 3**). This merits further studies that are underway using a multi-parent advanced generation intercross (MAGIC) approach, which has the potential to generate more recombinations.

Pyramiding of allelic combinations of flowering loci has the potential to induce earliness and further to combine earliness and yield capacity in spring barley. Gene-editing tools are now swiftly being developed, which have the potential to bypass past obstacles such as difficulty in breaking the linkage disequilibrium for loci located close to the centromeric regions. Better knowledge of epistatic effects of these genes with other loci in the flowering pathway such as *HvFT1* will enable breeders to fine-tune the flowering time of high yielding cultivars enabling a further expansion of cereal cultivation northwards.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, MZ286789-MZ286948.

AUTHOR CONTRIBUTIONS

MG: conceptualization, data analysis, methodology, writing original draft, and review and editing. TS: genetic analyses, data analysis, and writing original draft. ML: methodology and review and editing. TB: data analysis and review and editing. JH: conceptualization, genetic analyses, data analysis, methodology, validation, writing original draft, and review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.720238/full#supplementary-material

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