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Speed vernalization to accelerate generation advance in winter cereal crops

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Abstract

There are many challenges facing the development of high-yielding, nutritious crops for future environments. One limiting factor is generation time, which prolongs research and plant breeding timelines. Recent advances in speed breeding protocols have dramatically reduced generation time for many short-day and long-day species by optimising light and temperature conditions during plant growth. However, winter crops with a vernalization requirement still require up to 6–10 weeks in low-temperature conditions before the transition to reproductive development. Here, we tested a suite of environmental conditions and protocols to investigate if vernalization can be satisfied more efficiently. We identified a vernalization method consisting of exposing seeds at the soil surface to an extended photoperiod of 22 h day:2 h night at 10°C with transfer to speed breeding conditions that dramatically reduces generation time in both winter wheat (*Triticum aestivum*) and winter barley (*Hordeum vulgare*). Implementation of the speed vernalization protocol followed by speed breeding achieved up to five generations per year for winter wheat or barley, whereas only two generations can be typically completed under standard vernalization and plant growth conditions. The speed vernalization protocol that we developed in this study has great potential to accelerate biological research and breeding outcomes for winter crops.

Keywords

Speed breeding, speed vernalization, photoperiod, temperature, wheat, barley, crops, breeding

Introduction

Vernalization is the requirement for a prolonged period of cold before certain plants can transition from vegetative to reproductive development. Vernalization thus coordinates a plant's development with its environment. In agriculture, vernalization maximises the growing season of a crop by enabling autumn sowing without the risk of plants transitioning to reproductive development and becoming damaged by winter conditions (Kim et al., 2009; Xu and Chong, 2018). The longer growth duration improves crop productivity and is a common feature of many wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) cultivars grown throughout the high-yielding regions of Northern Europe, the United States, Asia, and the South Pacific. However, retaining vernalization in elite germplasm comes at a cost for generation turnover in breeding programmes. To date, the agronomic and academic standard cereal vernalization protocol entails 6–10 weeks at a low temperature of 2–6°C under short-day (8-h-light: 16-h-dark) photoperiod, where the lighting conditions are low light intensity (Luo

and He, 2020; Xu and Chong, 2018). These conditions have been selected as they best mimic the natural late autumn/winter, which has traditionally been believed to be the time during which vernalization occurs. In support of this, the major cereal vernalization genes *VERNALIZATION* (*VRN*) *1, 2* and *3* show expression patterns which alter with respect to the winter-type conditions. *VRN1,* a floral promoter, is activated in response to cold temperatures and *VRN2,* a floral repressor, is activated by long-day photoperiods (16-h light: 8-h dark) (Trevaskis, 2003; Yan, 2003; 2004). Thus, *VRN1* and *VRN2* are activated and repressed respectively under the artificial vernalization conditions which enable the post-vernalization increase in the expression of *VRN3*, which is also called *FLOWERING LOCUS T 1- like* (*FT1-like). VRN3* is believed to function in a similar way to the *Arabidopsis thaliana FT1,* integrating the environmental signals and coordinating the transition from vegetative to reproductive development.

Interestingly, there is an increasing amount of evidence which is suggesting that vernalization will proceed under less-classical conditions (Dixon et al., 2019; Duncan et al., 2015; Yan, 1999). In cereals, the optimal vernalization temperature for certain cultivars is between 8-14°C and vernalization has been observed to proceed at these warmer temperatures in more modern elite wheat as well (Dixon et al., 2019; Yan, 1999). In Arabidopsis, the same has been observed, where plants are fully vernalised under warmer temperatures experienced in the autumn (Duncan et al., 2015; Hepworth et al., 2020). This suggested that the vernalization conditions which are predominantly used in academia and industry may be suboptimal.

Here, we optimised the environmental conditions during vernalization and obtained a substantial reduction in the generation time for allohexaploid winter wheat and diploid winter barley. This new soil-surface protocol uses an extended photoperiod (22-h-light: 2-h-dark) and warmer temperatures (8–10°C) than typical vernalization treatments and was effective across a genetically diverse set of germplasm. This 'speed vernalization' protocol will accelerate the pace of research, training, and pre-breeding and breeding outcomes for winter cereal crops.

Results and Discussion

Vernalization proceeds under warmer temperatures

Due to vernalization, crop improvement programmes that focus on developing winter cultivars do not fully benefit from recent advances made via speed breeding, in which the photoperiod is extended to a 22-h daylength and plants are grown at 22°C during this period (Supplementary Table 1) (Hickey et al., 2019; Watson et al., 2018). Vernalization is considered a winter response, and artificial vernalization conditions reflect that with a standard protocol of 6–10 weeks at 2–6°C under a short-day, 8-h-light:16-h-dark, photoperiod (Dixon et al., 2019;

Kim et al., 2009). We tested vernalization efficiency at warmer temperatures and under short-day photoperiods (Supplementary Fig. 1a-f). As the vernalization response is quantitative (i.e., up to the point of vernalization saturation, increasing amounts of vernalization will lead to an acceleration in flowering time), the rate and point of completion of vernalization can be assessed by transferring vernalising plants to floral inductive conditions and scoring flowering time. To investigate if *VRN-A1* copy number was regulating the vernalization temperature required, we tested wheat cultivars with weak (e.g., cv. Claire, one copy of *VERNALIZATION1* (*VRN-A1*)), moderate (e.g., cv. Buster, two *VRN-A1* copies), and strong (e.g., cv. Charger and Hereward, three *VRN-A1* copies) vernalization requirements (Diaz et al., 2012). For all cultivars tested, vernalization completed efficiently following 6 weeks at 10°C or 14°C (Supplementary Fig. 1a-b), with warmer temperatures leading to the development of large vegetative meristems (Supplementary Fig. 1c-f). Our results support evidence from *Arabidopsis* in which vernalization completes in the autumn (Hepworth et al., 2018), suggesting that artificial vernalization conditions using low temperatures of 4–6°C may not be necessary. Here, raising the vernalization temperature to 10°C for 6 weeks met all vernalization needs of all tested cultivars, even those with long vernalization requirements. Importantly, these new conditions of 10°C under an 8-h-light:16-h-dark photoperiod, which we refer to as warm regular vernalization (wRV) (Table 1), can be supported by most controlled growth chambers.

Vernalization proceeds efficiently under extremely long-day lengths

Building on this, we were curious if there were opportunities for further optimisation. Short-day photoperiods are typically used for artificial vernalization of cereals as they repress the expression of the long-day-activated flowering repressor locus *VRN2,* which comprises two closely related genes *ZCCT1* and *2* [for *Zinc domain and CONSTANS, CONSTANS-LIKE, TOC1*] (Yan, 2004). However, there is increasing evidence that pre-vernalization repression of flowering in cereals is a multigenic response *(Greenup et al., 2010; Xie* et al*., 2021)*, prompting us to hypothesise that *VRN2* may not be a limiting factor. To test this possibility, we vernalised plants at 10°C under a 22-h-light:2-h-dark photoperiod (speed vernalization: SV) before transfer to speed breeding (SB) conditions (Watson et al., 2018) (SB: 22 h light:2 h dark, 22°C:17°C cycles or constant 22°C) (Table 1, Fig. 1a). As with wRV, meristems remained vegetative during SV (Supplementary Fig. 1g-n), and the plants went on to produce fully developed, fertile spikes. As expected, we observed a vastly accelerated generation time when SB followed vernalization, rather than regular glasshouse conditions (RG 20°C; 16 h light:8 h dark) and we did not see any examples of devernalization (Fig.1, Supplementary Fig.1). We observed a reduction in generation time for cultivars subjected to SV compared to wRV when plants were transferred to SB, particularly when considering the shorter

vernalization period of 2 weeks (Fig. 1). However, as with regular vernalization, we observed genotypic variation (Fig. 1, Supplementary Table 2). In cv. Claire, an acceleration in generation time was observed following 2 or 6 weeks of vernalization but not 4 weeks when comparing wRV and SV (Fig 1b). For cv. Charger, 4 weeks of the SV treatment accelerated flowering from non-flowering under wRV to 125 days under SV when both were transferred into SB (Fig. 1c). However, this acceleration still meant that the cv. Charger generation time was longer than that of a cultivar with a lower vernalization requirement (e.g., cv. Claire). The variability in the response suggested that *VRN-A1* copy number may be important in SV as it is under regular vernalization (Diaz et al, 2012; Dixon et al, 2019), so we were interested in identifying ways to improve our protocol to mitigate this.

Seed surface vernalization enables further efficiencies in generation time

We explored other parameters to increase the universal nature of the vernalization protocol. Under standard practice, plants are transferred to vernalization following 1–2 weeks of growth under regular glasshouse conditions (RG 20°C; 16 h light:8 h dark) to allow seedling establishment (Xie et al., 2021). Our SV protocol uses germination and growth under 22-h-light:2-h-dark photoperiods, so seedlings grow faster than with wRV. Therefore, to limit the extent of initial seedling growth, we tested if seedlings responded efficiently without pre-growth in the glasshouse. Accordingly, we subjected seeds to four treatments: T1) germination and growth at 4°C in the dark, T2) germination in SB before transfer to SV, T3) SV with the seed buried in soil, and T4) SV with the seed on the soil surface, hereafter referred to as speed green vernalization (SGV) (Table 1, Fig. 1f, Supplementary Table 3). Unexpectedly, vernalization was most efficient when seeds were placed on the soil surface and exposed to light under SV conditions (Fig. 1g), which significantly reduced the time to flowering by an additional 8 days compared to the other conditions (Duncan's multiple test, $\alpha = 0.05$) for cv. Keumgang (one *VRN-A1* copy). To optimise the protocol, we tested a suite of durations (between 1 – 6 weeks, at 8-10°C) and temperatures (6 - 12°C, for 4 weeks) of SGV conditions including for the Korean winter wheat cv. Keumgang and the American winter wheat cv. Sturdy (one *VRN-A1* copy). These experiments confirmed that 8–10°C is the most efficient and reliable vernalization temperature (Fig. 1j-k, Supplementary Tables 4 and 5). This result revealed a seed-based aspect of the vernalization response in cereals that is similar to dormancy in dicots (Chen et al., 2014). The same method (SGV) was also successful in cultivars with a strong vernalization requirement (e.g., cv. Hereward and Charger) and reduced generation time by at least 4 weeks compared to SV or wRV and transfer to SB (Fig. 1b-i). Importantly, SGV followed by SB (hereafter SGV–SB) conditions reduced the duration of vernalization needed, with 4 weeks at 10°C being optimal, although there was genotypic

variation for this; therefore, our protocol enables a higher throughput of plants through vernalization compared to RV and SV.

For use in academic and industrial breeding programmes, the SGV–SB protocol should support usual plant development. Therefore, we measured multiple plant growth parameters, obtained normal seed set, and observed standard plant development, although plant height was slightly reduced (Supplementary Fig. 2). We also tested the effectiveness of the SGV–SB protocol on a wheat diversity panel regularly used in wheat breeding programmes in Korea. These cultivars varied in the allelic composition at the *VRN1* locus (Supplementary Table 6). Of the 51 winter cultivars in the panel, 45 were fully responsive to SGV–SB conditions (Supplementary Table 6). The non-responsive cultivars were recessive for each of the *VRN1* genes (on the A, B and D genome) except for Minihardi that carries a *Vrn-A1b* allele. However, this cannot be the cause of the lack of response as multiple other lines with the same alleles at the *VRN1* locus were fully responsive to SGV–SB (Supplementary Table 6 and cv. Charger, Hereward and Claire, Fig. 1). Potentially, variation in light intensity may influence the responsiveness (Supplementary Fig. 3). Overall, our data demonstrate that vernalization on the soil surface imposed by the SGV–SB protocol meets and reduces the vernalization requirement and generation time for many winter wheat cultivars tested and is effective even on cultivars that traditionally have a longer vernalization requirement.

Seed-surface vernalization is also efficient for winter barley

Given the similarity between the vernalization response of wheat and barley, we hypothesised that SGV–SB may accelerate generation time in winter barley as well. Accordingly, we subjected 60 diverse winter barley cultivars originating from 34 growing regions or countries to three treatments: RV–RG, RV–SB, and SGV–SB (Supplementary Fig. 4a, b). The 60 winter barley accessions evaluated were largely representative of the genetic diversity in a global panel comprising 806 accessions sourced from the Australian Grains Genebank (Supplementary Fig. 4a). Following genomic analysis of the panel using 9,221 SNPs, very little population structure was related to ‘winter’ or ‘spring’ type classification (Supplementary Fig. 4a). This suggests that farmers and breeders have historically selected and used germplasm with a range of vernalization requirements to develop locally adapted varieties. We recorded days to flowering in each experiment (Fig. 2a, b). Notably, all cultivars flowered and produced viable seeds under all conditions. The average time to flowering observed across the three treatments was significantly different (Tukey’s multiple comparison, $P < 0.05$). Under SGV–SB, the entire population flowered substantially earlier, on average after 50 days compared to 92 days under RV–RG and 68 days under RV–SB (Fig. 2a). Therefore, in contrast to wheat, the collection of winter barley cultivars was completely responsive to the SGV–SB protocol.

Despite the dramatic response displayed by the winter barley accessions, time to flowering was correlated across all three treatments (Supplementary Fig. 4c). Overall, these results highlight the utility of the SGV–SB protocol to substantially reduce generation time for diverse winter barley germplasm.

Rapid generation advance for winter cereals is now similar to spring cereals

Crossing and the subsequent development of genetically stable or inbred lines are routine practices in both breeding and research programmes. However, these techniques are particularly time consuming for populations derived from winter × spring and winter × winter crosses, as each generation must be vernalised to avoid unintended selection against the vernalization mechanism. To test the SGV–SB protocol for use in population development, we crossed cv. Jokyung (spring) and cv. Joongmo2008 (winter) wheat cultivars in the field in Korea during the 2018–2019 winter season. Throughout the subsequent 15 months, we reached the F5 generation, with each generation taking an average of 82.4 days (Fig. 2c, Supplementary Table 7). The SGV–SB protocol considerably reduced the variation in days to flowering for populations derived from winter × spring and winter × winter crosses (Fig. 2d; Supplementary Fig. 5). Early and more synchronous flowering across segregating or diverse germplasm can facilitate more efficient crossing and rapid generation times. We harvested at least 25 seeds from each F5 plant, which is sufficient to bulk seed in the field and subsequent evaluation. Using a projected timeline for plant generations (Fig. 2e-f), we highlight the opportunity to increase the number of plant generations within a 12–18-month period for both spring and winter wheat and barley. Impressively, the SGV–SB protocol applied to some winter cultivars reached generation turnover times similar to those of spring cultivars. Furthermore, the seed surface vernalization is extremely high-throughput, with a density of up to 1,709 seeds per m^2 being treated when utilising 128-cell seed trays.

Speed vernalization alters expression of key genes in the vernalization and flowering pathway

Our optimised vernalization conditions (SV and SGV) that lead to a reduction in overall generation time challenge our current understanding of the vernalization mechanism itself, where short days reduce *VRN2* (a locus comprised of *ZCCT1* and *ZCCT2*) expression and cold temperatures activate *VRN1* expression (Dixon et al., 2019). Therefore, we investigated how vernalization genes responded during vernalization and if they differed between SV versus SGV (Fig. 3). The expression patterns of vernalization genes (primer details in Supplementary Table 8) followed the expected profiles in wRV–SB, using cv. Claire, with *VRN1* and *VRN3* (also named *FLOWERING LOCUS T-like1* (*FT1*)) transcript levels increasing during vernalization (Fig. 3a-b). Notably, *VRN1* and *VRN3* expression was lower under SV

compared to wRV conditions (Fig. 3a-c), indicating that these genes may not represent the exclusive route promoting flowering under SV. We observed a sustained repression of *ZCCT1* expression during and following SV when plants were transferred to SB, which was unexpected given the extended photoperiod of SV, which would be anticipated to promote *VRN2* expression (Fig. 3d). However, comparing the SV–SB conditions in cv. Hereward, which vernalised more rapidly with SGV protocol, *ZCCT1* steadily increased in expression, opposite to that in the SV condition (Fig. 3j-k). This result indicated that considering the *VRN2* locus as a single gene is potentially misleading for our understanding of the vernalization response and that an additional or alternative vernalization response is activated during SGV. To further understand the differences between SV and SGV at the molecular level, we also measured the expression of genes associated with floral regulation in wheat; the photoreceptor *PHYTOCHROME C* (*PHYC*), the low-temperature responsive gene *GIGANTEA* (*GI*) and a *CONSTANS-like gene* (*CO*) (Supplementary Fig. 6). These all showed different expression patterns between SV and SGV and so indicate that the SGV response is modifying genes that regulate flowering early in wheat plant development. Further examination of these responses may identify new breeding targets in the regulation of vernalization and flowering time in cereals.

A framework to reduce generation time for crops with a vernalization requirement

The time required for the introgression and stacking of novel alleles remains a bottleneck in the development of improved cultivars. One of the limiting factors is the vernalization requirement, which imposes a biological constraint on generation time. Here, we identify a method to reduce generation time for winter wheat and barley germplasm using alternative vernalization conditions. The protocols we developed identify the environmental parameters that can be further modified to account for local or genotypic variation in vernalization efficiencies and therefore offer a framework to universally reduce generation times in winter cereals. The approach could potentially be adapted to other winter crops (e.g., canola [*Brassica napus*]) or vegetables with a vernalization requirement to reduce generation time and accelerate breeding outcomes.

Methods

Plant materials

Wheat and barley cultivars used in this study are in Supplementary Table 9.

Evaluation of European winter wheat lines and gene expression (assessed at The University of Leeds, UK)

The conditions used to evaluate wheat (*Triticum aestivum*) lines included:

- *wRV–RG:* 8 h light:16 h dark 10°C into 16 h light:8 h dark 22°C
- *wRV–SB*: 8 h light:16 h dark 10°C into 22 h light:2 h dark 22°C
- *SV (and SGV) –SB*: 22 h light:2 h dark 10°C into 22 h light:2 h dark 22°C

Please note warmer conditions were used in wRV than are classically used in RV to enable direct photoperiod comparison for phenotype and gene expression analysis.

Seeds were germinated for 2 days in darkness at 4°C in 9-cm petri dishes that had a layer of filter paper saturated with 5 mL dH_2O. The germinated seeds were transferred to 3 x 3-cm cell pots of JIC cereal mix (Dixon et al., 2019) and placed under vernalization conditions in Snijders MICROCLIMA MC1000 cabinets. Plants were watered when required, and no additional nutrients were added. At 1- or 2-week intervals, plants were sampled for gene expression and apex analysis (see below), and three plants were transferred to glasshouse growth conditions for RG (PhytoLux Plessey; model ATTIS-7) and SB (Heliospectra; model MITRA). Plants were placed in cereal mix (as above) in 9 x 9-cm pots. Plants were sampled 1 and 2 weeks after transfer to SB conditions. Flowering time was recorded as half-ear emergence (Zadok scale 55). Plants that did not flower after 170 days were recorded as "non-flowering (NF)." A control group using the same four genotypes ($n = 10$, $s = 40$) was grown under constant SB conditions, and a representative for each genotype was imaged once the plant had reached maturity.

For the speed-green vernalization treatment, seeds were placed in 9-cm petri dishes containing filter paper and 5 mL dH_2O and kept in complete darkness at 4°C for 48 hours. Seeds were then placed on the soil surface (John Innes cereal mix) in a P24 seedling tray. Care was taken to press the seed into the soil surface while ensuring the seed remained uncovered and exposed to the light. Plant trays were watered from the base, and a spray bottle of dH_2O was used to mist the soil surface; particular care was taken misting the soil during the first week of growth, when the roots were anchoring into the soil. At set weekly intervals, plants were moved into SB conditions and flowering was recorded.

For the gene expression study, leaf samples from three plants per biological replicate and for three biological replicates ($n = 3$) were taken at each sampling stage. Samples were taken 1 h after lights on and flash-frozen in liquid nitrogen. To investigate gene expression during SV and SGV, leaf tissue was sampled at 1, 2, 3, 4, 5, 6, 7, and 8 weeks of growth under vernalization conditions. The tissue was lysed using the TissueLyserLT (Qiagen) with 3mm steel ball bearings and total RNA was extracted using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich) following the manufacturer's recommended protocol. RNA samples were treated using RQ1 RNase-Free Dnase (Promega), and first-strand cDNA synthesis primed

with Oligo dT was processed using SuperScript™ III Reverse Transcriptase (Invitrogen) and RNaseOUT™ (Invitrogen). The cDNA was diluted (1:10), and quantitative reverse transcription PCR (RT-qPCR) was performed using the CFX96™ Thermal Cycler (Bio-Rad) with the following conditions: 95°C for 5 minutes, 39 cycles of 95°C for 10 seconds, 60°C for 30 seconds; followed by a melt starting at 65°C for 5 seconds, increasing in 0.5°C increments to 95°C and GoTaq® Master Mix (Promega). Primers used are provided in Supplementary Table 8. Expression levels of the genes of interest were calculated relative to TraesCS5A02G015600 following the $2^{\wedge -\Delta CT}$ format (where ΔCT = *GOI* CT – TraesCS5A02G015600 CT).

Apex samples: three plants were dissected for each apex sample (n = 3) and imaged on a Keyence microscope, and apex length was measured using ImageJ (Schneider, 2012).

Evaluation of Korean winter wheat cultivars and breeding application (assessed at RDA, S. Korea)

The conditions used to evaluate wheat (*Triticum aestivum*) lines included:

- wRV–SB: 8 h light:16 h dark 10°C into 22 h light:2 h dark 22°C:17°C
- SGV–RG: 22 h light:2 h dark 10°C into 16 h light:8 h dark 22°C:17°C
- SG*V–SB*: 22 h light:2-h dark 10°C into 22 h light:2 h dark 22°C:17°C

To optimize the vernalization treatment method, a series of experiments were performed. Seeds were germinated in 9-cm petri dishes containing 10 mL of water and at 4°C for 3 days under dark conditions. Then, seeds were transferred to 25°C until they reached the growth stage 07 and about 0.2-mm coleoptile length (Tottman, 1987). For all experiments, each square pot (7(L) × 7(W) × (7H)) used was filled with 245 mL of soil, which is a mixture of paddy rice soil (Punong, Korea) and horticulture soil (Seoul-Bio, Korea) at a 2:1 ratio. To find an optimum vernalization treatment method, four cold treatment methods were applied using the Korean winter variety Keumgang as follows: T1) seed vernalization, T2) green vernalization, T3) speed-green vernalization with covering soil, and T4) speed-green vernalization without covering soil about 1.5 cm in depth. Regarding the seed vernalization method (T1), germinated seeds were placed in a 4°C refrigerator for 4 weeks without light and cultivated under speed breeding (SB) conditions. For green vernalization (T2), plants initially placed under SB for 1 week were further grown under 22-h-light:2-h-dark photoperiod cycles at 8°C for 4 weeks. Concerning the speed-green vernalization with (T3) and without (T4) covering soil (SGV), germinated seeds were sown and grown at 8°C for 4 weeks under 22-h-light:2-h-dark cycles. LED lights (red 8: blue 3: white 2, Estech LED, Korea, Supplementary Fig. 2b-c) in all the vernalization conditions were set to 100 µmol m^{-2} s^{-1} of illumination. All plants were then

transferred to SB (500 µmol m^{-2} s^{-1} of light) to evaluate the time to flowering after vernalization treatment; *n* = 8 plants for each evaluation. Spectral measurement of light composition was performed using the LI-250A light meter (LI-COR Biosciences, USA).

To optimize the cold treatment protocol for speed vernalization, temperatures ranging from 6–12°C were applied for 1–6 weeks under SGV condition. Six plants were then transferred to SB conditions to examine days from germination to heading. The growth stages (GS32 and GS59) were recorded as reported by (Tottman, 1987), and plant height was measured from the ground to the bottom of the spike where it meets the peduncle.

To investigate days from germination to heading of wheat genetic resources, germinated seeds were placed in the cell of a 72-cell tray (34 mL/cell) and cultivated under the SGV condition. Plants were grown under SGV for 4 weeks, then transferred to SB and observed until heading (GS59), *n* = 4.

To investigate the ability to scale-up the approach, SGV was conducted under a range of planting densities that supported high-throughput vernalization. Two seed trays were evaluated: a 72-cell seed tray, yielding a density of 466 plants/m^2, and a 105-cell tray, yielding a density of 680 plants/m^2.

To evaluate the actual application of SGV in a breeding programme, 10 F1 seeds were obtained from a cross derived between cv. Jokyung (spring wheat) and cv. Joongmo2008 (winter wheat). For each generation from F1 to F5, germinated seeds were placed into 72-cell trays (1 seed per cell, 34 mL/cell) and cultivated under the SGV condition. Plants were grown under SGV for 4 weeks, followed by transfer to SB until harvest. After harvest, seeds were dried at 35°C for 4 days, then they were imbibed by placing on moistened filter paper and chilled at 4°C for 3 days to break dormancy. For the F6 generation, 25 seeds of each line were planted in the field at the National Institute of Crop Science, Miryang Korea (35.3° N; 128.5° E).

For identification of *VRN*1 allelic variation, genomic DNA was extracted from fresh leaves with DNA extraction buffer (Biosesang, Korea) according to the manufacturer's instructions. The total PCR reaction volume of 30 µL contained 25 ng of template DNA, 0.5 µM of each primer, 2.5 µL of 10mM dNTP, 3.0 µL of 10X buffer and 0.3 µL of Taq polymerase (Genetbio, Korea). The amplification program parameters were 94°C for 10 minutes for initial denaturation followed by 40 cycles of 94°C for 45 seconds, 45 seconds at each annealing temperature, and 72°C for 1 minutes. Amplified PCR products were separated on a 3% agarose gel and visualized using the G:Box gel documentation system (Syngene, Cambridge, UK). The

sequence of primers and sizes of the PCR-amplified products with the markers are listed in Supplementary Table 10 (Fu et al., 2005; Whittal et al., 2018).

Evaluation of diverse winter barley accessions (assessed at The University of Queensland, Australia)

The conditions used to evaluate the panel of barley (*Hordeum vulgare*) lines included:

- *RV–RG*: 8 h light:16 h dark 6°C into 12 h light:12 h dark 22°C:17°C
- *RV–SB*: 8 h light:16 h dark 6°C into 22 h light:2 h dark 22°C:17°C
- *SGV–SB*: 22 h light:2 h dark 8°C into 22 h light:2 h dark 22°C:17°C

The diverse barley accessions were evaluated in three experiments: 1) regular vernalization and regular glasshouse (RV–RG), 2) regular vernalization and speed breeding (RV–SB), and 3) speed green vernalization and speed breeding (SGV–SB). Under regular vernalization conditions, plants received a standard vernalization treatment at 6°C for 6 weeks under 8-h-light:16-h-dark photoperiod cycles. For speed green vernalization (SGV), plants were exposed to 8°C for 4 weeks under 22-h-light:2-h-dark cycles. Vernalization was performed in a fully enclosed walk-in growth cabinet fitted with LED growth lights (Heliospectra, model E602G). For the regular vernalization (RV), seeds were sown directly into 100-cell trays (18 mL per cell), covered with UQ23 potting mix (Ghosh et al., 2018), watered, and moved into the vernalization chamber. For speed green vernalization (SGV), seeds were pre-germinated at 22°C in petri plates until ~1 cm of emerging radicle was visible and then placed onto the surface of the potting mix. Three seeds per accession were transplanted into a single cell of the tray for vernalization. To retain moisture in the cells during vernalization, the 100-cell trays were placed inside a sealed bottom tray with two to three small drainage holes. Trays were lightly watered daily during the vernalization process.

After vernalization, the bottom trays were removed and filled with UQ23 potting mix and Osmocote® slow release fertiliser (at a rate of 2 g per litre) to provide developing plants with sufficient media and resources. For the regular glasshouse conditions, plants were grown in a temperature-controlled glasshouse (22:17°C, light:dark) under a natural 12-h diurnal photoperiod. For the speed breeding treatment, trays of barley plants were moved to a temperature-controlled glasshouse (22:17°C, light:dark) fitted with Heliospectra LEDs using a 22-h-light:2-h-dark photoperiod (Ghosh et al., 2018). The day of anthesis for each accession was recorded as the first spike to reach awn-peep stage (GS49).

Genotyping was performed to benchmark the genetic diversity within the 60 winter barley accessions examined in this study. The 60 accessions were selected from a panel sourced from the Australian Grains Genebank Collection (AGG) comprising 806 diverse accessions. The panel was genotyped using the Illumina Infinium 40K XT SNP chip assay (InterGrain and AgriBio-Victoria), which generated 12,599 SNP markers. Polymorphic markers with known chromosome positions were used to investigate the genetic diversity of the barley accessions (9,221 high-quality SNP markers with <10% missing data and <10% heterozygosity; and 737 barley lines with <10% missing values). To investigate the population structure relating to either facultative, spring or winter type classifications, we calculated the pairwise Roger's distances between the accessions using 'SelectionTools' (downloadable at http://population-genetics.uni-giessen.de/~software/) implemented in R. Principal coordinate analysis based on the Roger's genetic distance matrix and k-means clustering was performed and plotted using ggplot2 (Wickham, 2016) in R.

Variation in DTA for the barley accessions in each vernalization treatment was visualised in the form of density plots, generated using ggjoy package in R. To determine if DTA differed across the three treatments, one-way analysis of variance (ANOVA) was performed. Tukey's multiple comparison test (Tukey's HSD test) was then performed to evaluate the effect of each treatment on DTA. HSD test applies appropriate adjustments to the mean for each treatment suitable to multiple testing (Rogan, 1977). The analysis was performed using Agricolae package in R. To investigate the relationship between barley flowering behaviour across vernalization treatments, the Pearson's correlation coefficient (r) was calculated for DTA. The degree of correlation was also tested for significance (P-value; $\alpha = 0.05$) (Supplementary Fig. 4). The analysis was performed using corrgram and corrplot packages in R.

Author contributions

L.T.H., D.S. and L.E.D. conceived and supervised the project. J.H.L., L.T.H., D.S. and L.E.D. designed the experiments. J.K.C. K.O.C., D.H. and K.M.K. investigated flowering time of wheat. S.A. and E.D. investigated flowering time of barley. J.K.C, H.P., S.M.L., Y.K. and J.M.K. developed wheat breeding materials. J.K.C., K.O.C. and S.W.K. analysed the data. L.T.H., D.S. and L.E.D. wrote the manuscript. All the authors discussed the results and contributed to the manuscript.

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Supplemental information

Document S1. Supplemental Figures 1–6 and Supplemental Table 1-10

Tables

Table 1 Summary of the environmental conditions

Abbreviation	Defined abbreviation	Condition
RV	Regular Vernalization	8 h light:16 h dark 4-6°C
wRV	warm Regular Vernalization	8 h light:16 h dark 10°C
SB	Speed Breeding	22 h light:2 h dark 22°C:17°C or constant 22°C
RG	Regular Glasshouse	16 h light:8 h dark 22°C:17°C or constant 22°C
SV	Speed Vernalization	22 h light:2 h dark 10°C (seed buried)
SGV	Speed Green Vernalization	22 h light:2 h dark 10°C (seed on the surface)

Figure legends

Figure 1 Identifying speed vernalization conditions for wheat (*Triticum aestivum*)

Speed (green) vernalization (SV (SGV)) combined with speed breeding (SB) accelerates winter wheat life cycles. **a**. Scheme of SV/SGV–SB conditions. Comparison of flowering time from germination for **b**. cv. Claire and **c**. cv. Charger between wRV, SV, and SGV treatments and transfer to SB conditions following the vernalization duration indicated. Total time to flowering is shown (including vernalization duration); n = at least 6. Fastest generation time following wRV, SV, and SGV indicated by the time the first plant flowered following transfer to SB for **d**. cv. Claire and **e**. cv. Charger. **f**. Comparison of seed treatments with example images taken when the first plant flowered under T4 conditions for the winter wheat cv. Keumgang. **g**. Flowering time following the four seed treatments (T1-4 in f) in cv. Keumgang; n = 7. **h**. Comparison of plant development between SV and SGV. **i**. Comparison of flowering time for cv. Hereward between wRV and SGV; n = at least 6. Flowering times for different durations (1 – 6 weeks, at 8-10°C) **j**. and temperatures (6 - 12°C, for 4 weeks) **k**. for cv. Keumgang under SGV. Significance is shown according to Student's t-test $P < 0.05$. SV (speed vernalization), SGV (speed-green vernalization), wRV (warm regular vernalization), SB (speed breeding), and vernalization duration are included in days to flowering. NF = non-flowering.

Figure 2 Speed vernalization conditions for barley (*Hordeum vulgare*) and cereal population development

Flowering time responses of diverse winter barley accessions following various vernalization conditions. **a**. Density plots displaying days to anthesis (DTA) for 60 diverse winter barley accessions evaluated using three treatments: 1) speed vernalization (at 8°C) and speed breeding conditions (SGV–SB), 2) regular vernalization and speed breeding conditions (RV–SB), and 3) regular vernalization and regular glasshouse conditions (RV–RG). **b**. Example image of barley population following SGV–SB. **c**. Population development timeline for winter x spring wheat population over six generations and **d**. flowering comparison between SB and SGV–SB for spring x winter population. **e.** An example breakdown of growth cycle under SGV–SB conditions for the same winter x spring cross in **c** and **d**. **f.** Projected generation times for different cereal types and growth conditions: A = SB Spring barley (e.g., cv. Commander, Golden Promise), B = SB Spring wheat cv. Suntop, Cadenza, C = SGV–SB Winter barley, D = SGV–SB Wheat winter x spring (using data from d), E = SB wheat winter x spring (using data from d), F = SGV–SB winter wheat cv. Claire, G = SV–SB winter wheat cv. Claire, H = SGV–SB winter wheat cv. Charger, I = SV–SB winter wheat cv. Charger, J = wRV–RG winter wheat cv. Charger, and K = wRV–RG cv. Claire. Spring generation times calculated from published data (Watson et al., 2018). SV (speed vernalization), SGV (speed-green vernalization), wRV (warm regular vernalization), SB (speed breeding).

Figure 3 Vernalization-related gene expression under SV and SGV conditions

In cv. Claire, **a**. Comparison of expression of *VRN1* between wRV (green) and SV (pink) conditions. **b**. Comparison of expression of *FT1-B* between wRV (green) and SV (pink) conditions. **c**. Expression of *VRN1* (green) and *FT1-B* (red) between 6 weeks of SV and then 6 weeks of SV and 1 week in SB. **d**. Expression of *ZCCT1* and *ZCCT2* between 6 weeks of SV and then 6 weeks of SV and 1 week in SB. Representative images following different vernalization treatments. For cv. Claire following SV–SB with 3 weeks **e**., 4 weeks **f**., and 5 weeks **g**. vernalization treatment. Comparison between wRV and SGV conditions for cv. Claire following 4 weeks vernalization and photographed at the same age **h**. and cv. Hereward following 6 weeks vernalization and photographed at the same age **i**. All conditions result in vernalised plants. Scale bar is 30 cm. In cv. Hereward, expression of *VRN1*, *ZCCT1*, *ZCCT2*, and *FT-1B* for **j**. SV and **k**. SGV. All show three biological replicates that each comprise at least three plants, with standard error of the mean. SGV (speed-green vernalization), wRV (warm regular vernalization), duration in weeks refers to vernalization duration experienced.

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Figure 1

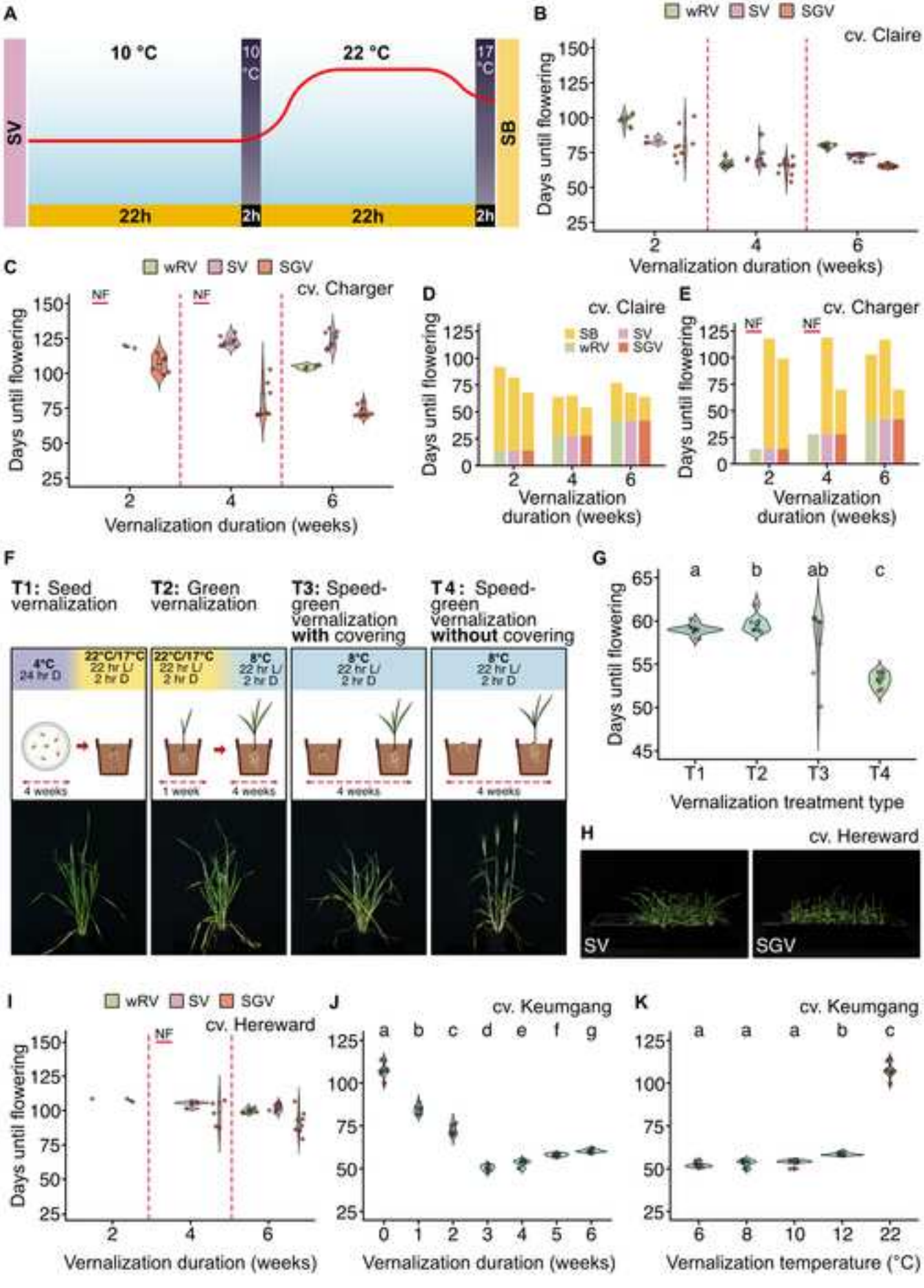

Figure 2

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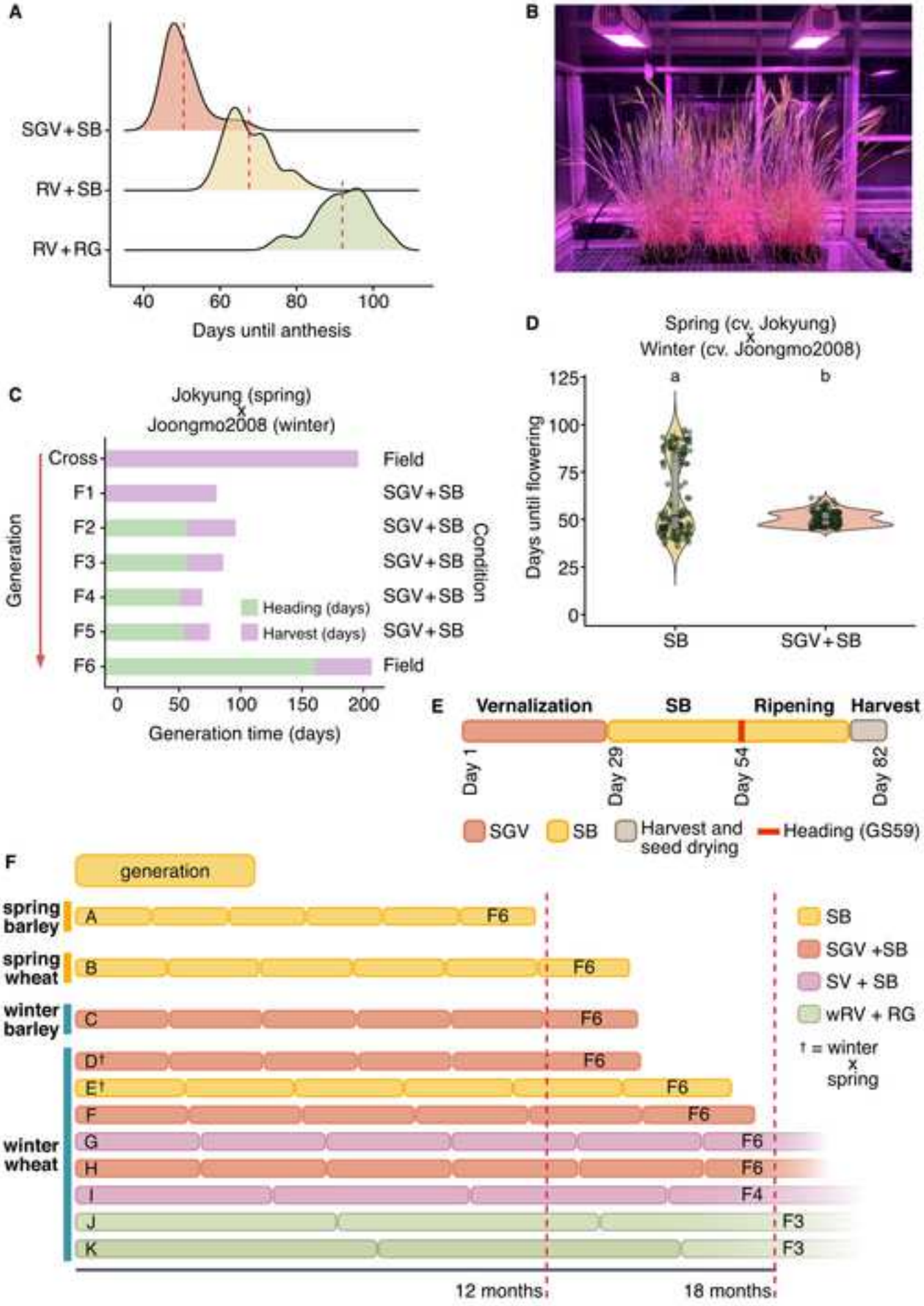
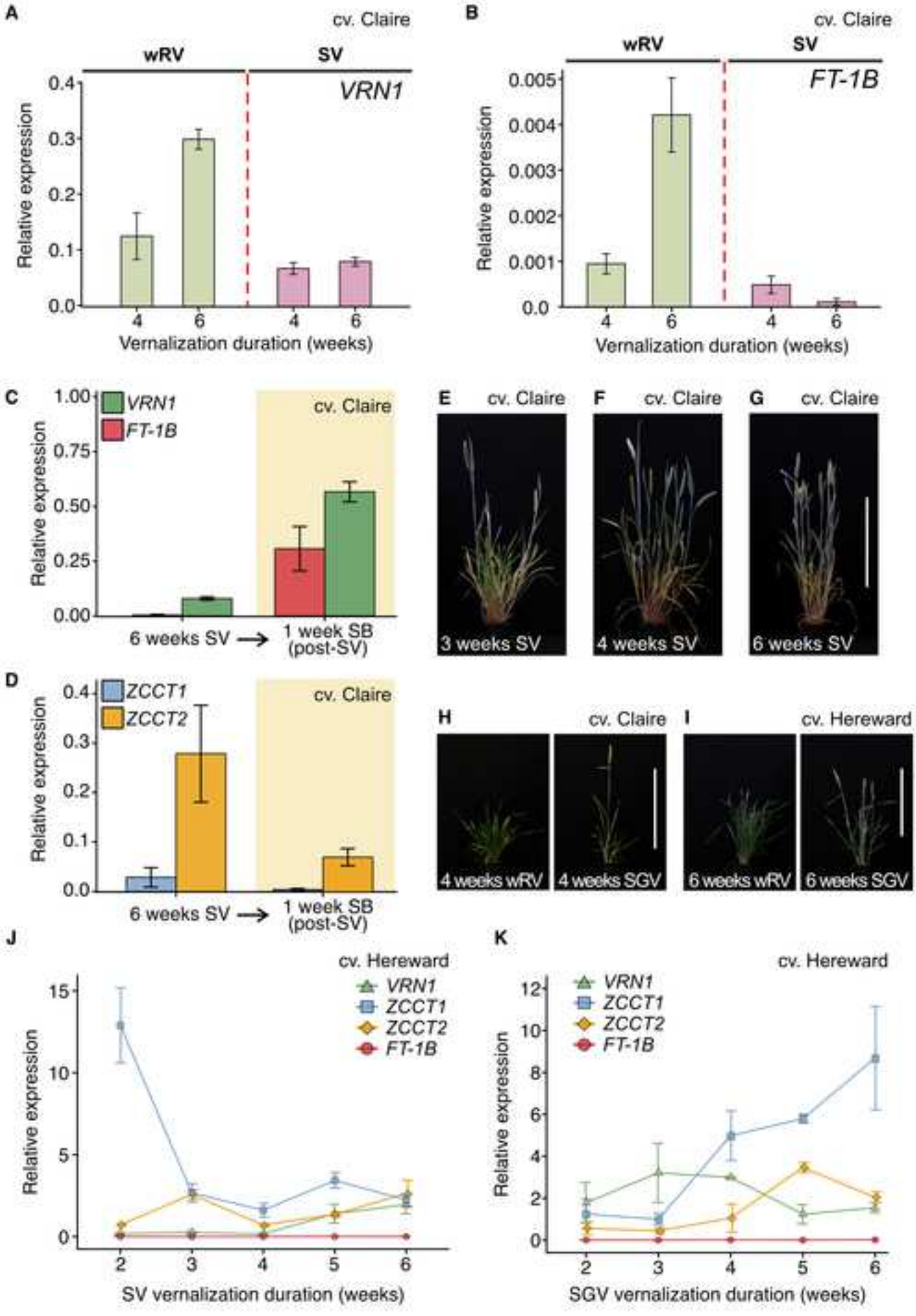


Figure 3



Supplementary Figures and Tables

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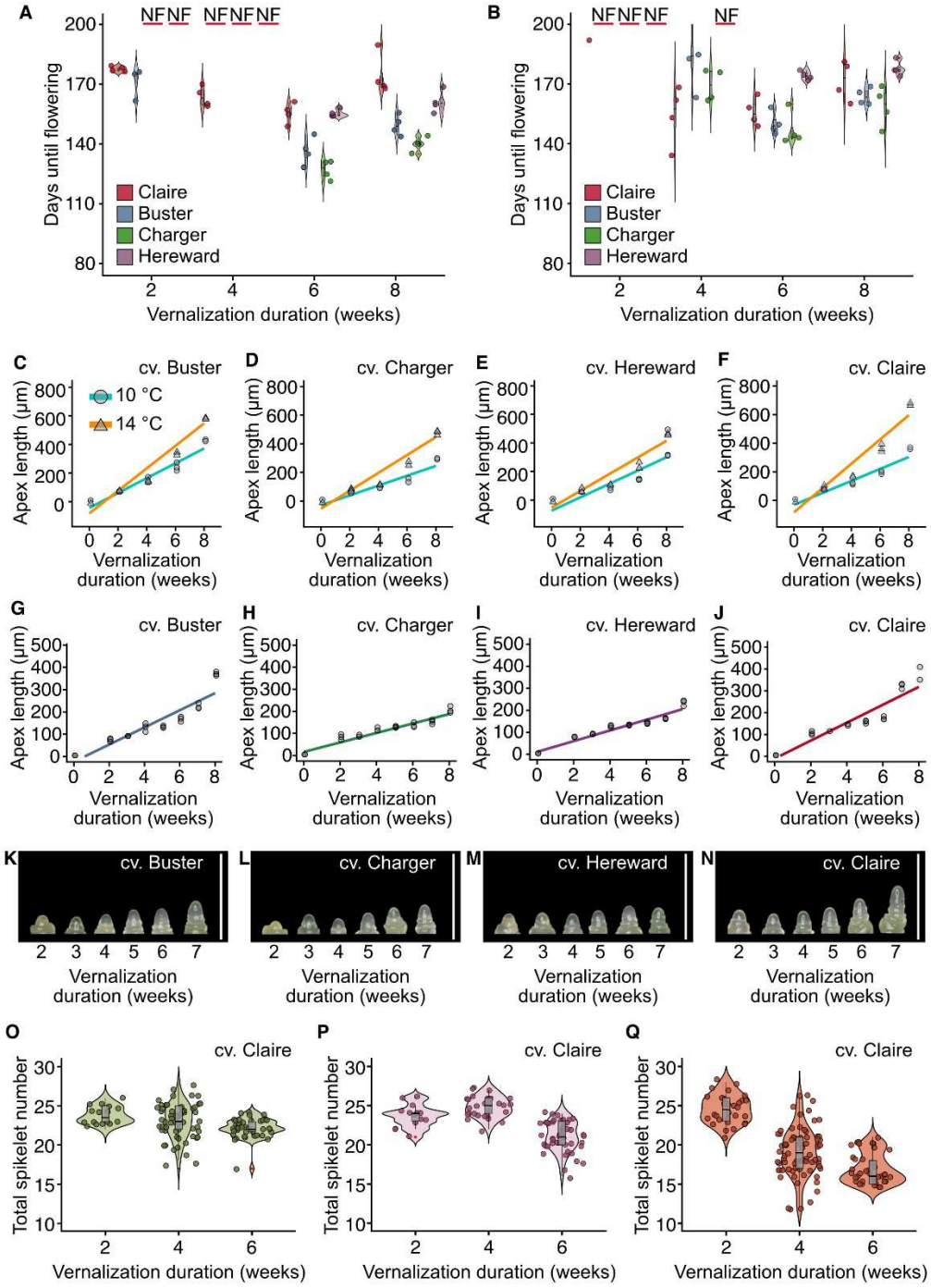
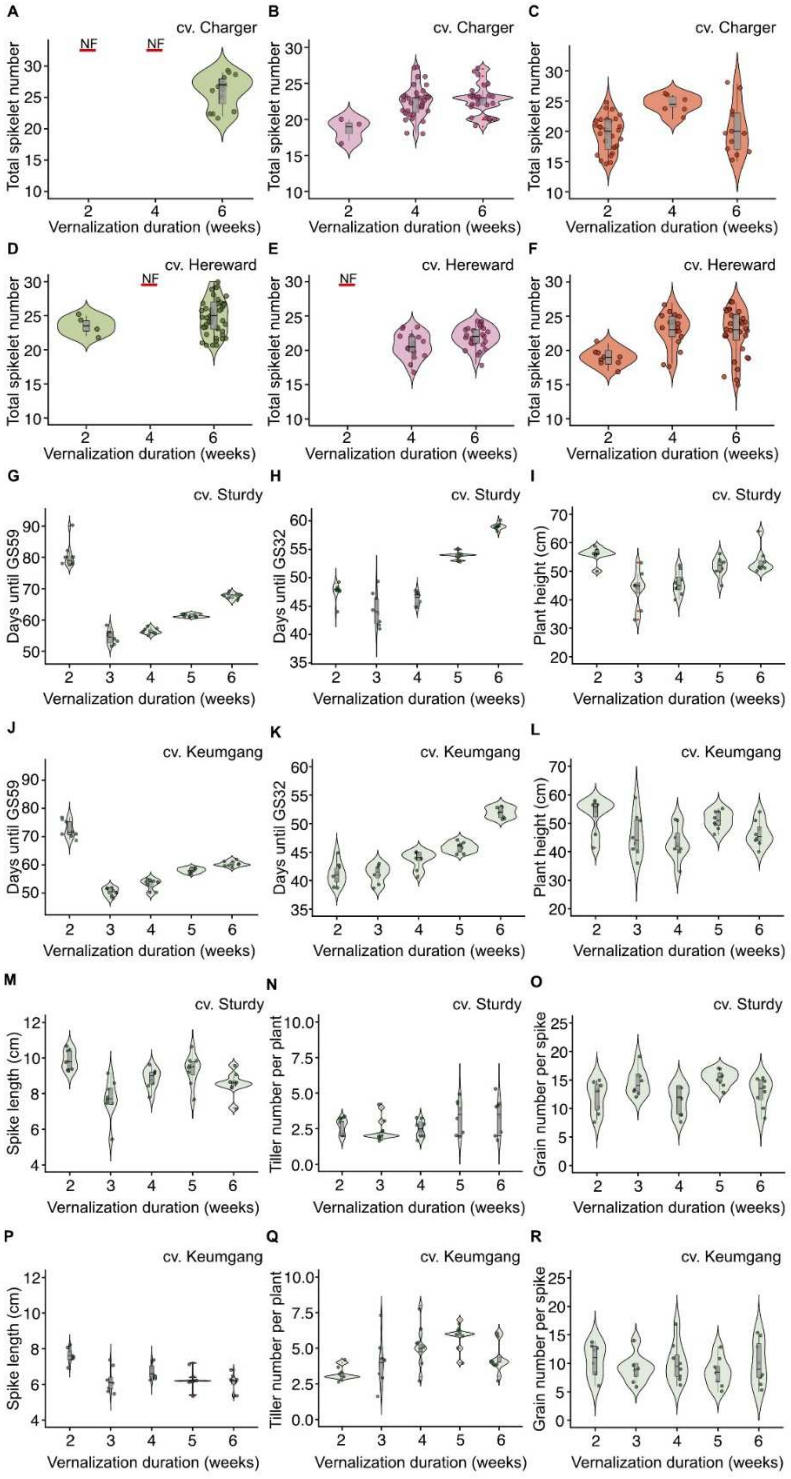
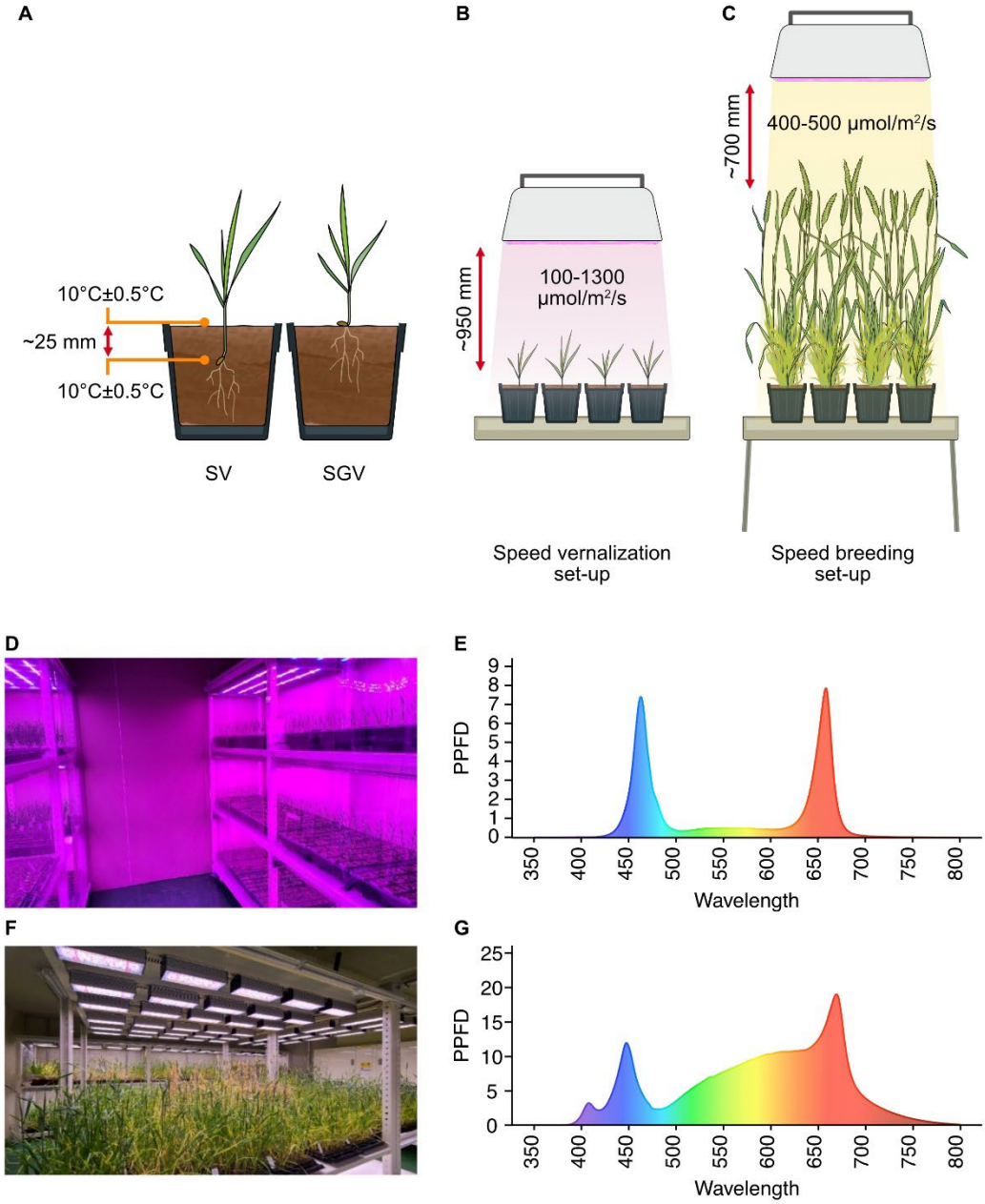
6 supplementary figures and 10 Tables



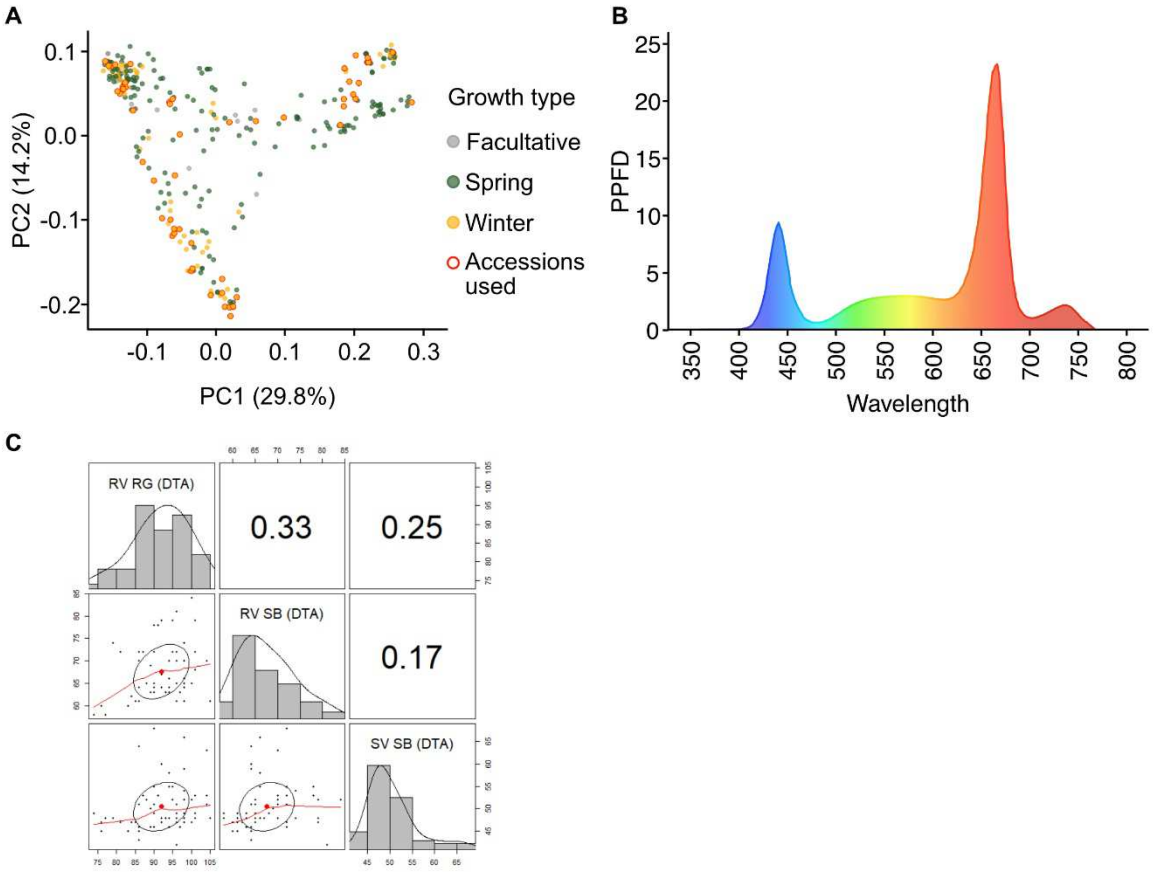
Supplementary figure 1: Plant development under different vernalisation temperatures for four European winter wheat cultivars. Days to flowering (GS59) for winter wheat cultivars Claire, Buster, Charger, and Hereward vernalising under **A**. 10°C or **B**. 14°C short-day (8 h light:16 h dark) photoperiod and transferred at the weeks indicated on the x-axis to long-day (16 h light:8 h dark) glasshouse conditions with constant 20–22°C. Days to flowering includes total number of days from germination to GS59/ear completely emerged. Vernalisation duration is included in the flowering time. *n* = at least 3, NF = No flowering by date indicated. **C** - **F** Apex lengths from the same conditions; *n* = 3. Apex length following SV (10°C, 22 h light: 2 h dark with seeds buried) for cultivars **G**. Buster, **H**. Charger, **I**. Hereward, and **J**. Claire; *n* = 3 with representative images between weeks 2 and 7 of SV for each cultivar, scale bar represents 1000 µm, for cultivars **K**. Buster, **L**. Charger, **M**. Hereward, and **N**. Claire.



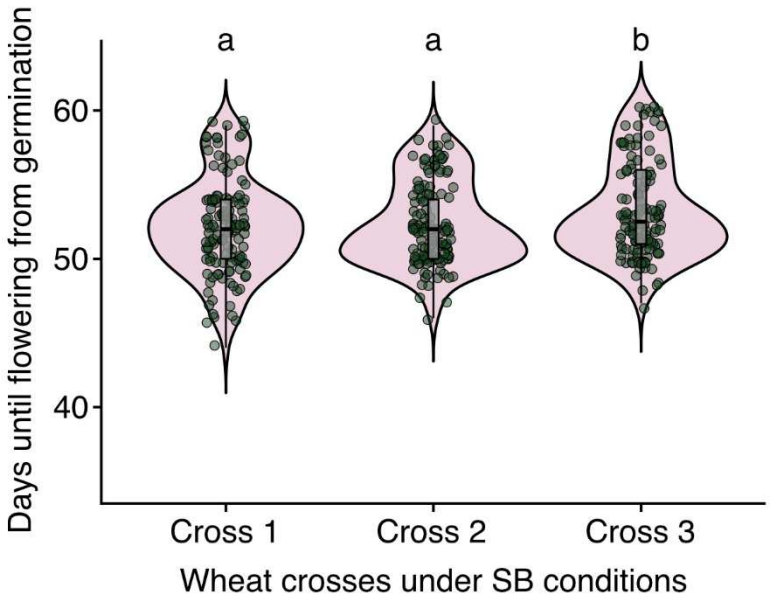
Supplementary figure 2: Spikelet counts from plants moved following 2, 4, or 6 weeks of vernalisation for cv. Charger for **A**. wRV–SB, **B**. SV–SB, and **C**. SGV–SB and cv. Hereward for **D**. wRV–SB, **E**. SV–SB, and **F**. SGV–SB, *n* = at least 8. Under SGV–SB conditions, wheat plants followed standard development shown over a 2- to 6-week vernalisation treatment for cv. Sturdy: **G**. GS59 from germination, **H**. days until GS32 from germination, **I**. plant height for cv. Keumgang, **J**. GS59 from germination, **K**. days until GS32 from germination, **L**. plant height for cv. Sturdy, **M.** Spike length, **N**. tiller number per plant, and **O**. grain number per spike; and for cv. Keumgang: **P**. spike length, **Q**. tiller number per plant, and **R**. grain number per spike, where *n* = 6. NF=Non-flowering.



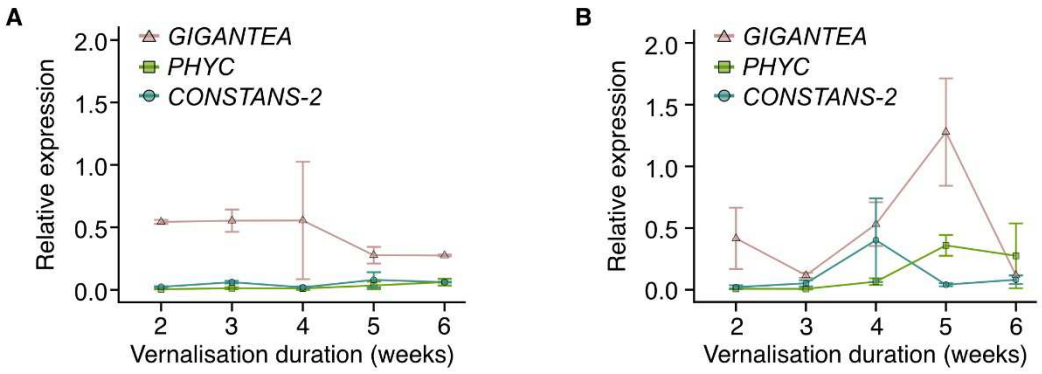
Supplementary figure 3. Light intensities and quality used under SV and SGV conditions. **A**. Comparison of plant position during SV (buried seed) and SGV (seed on the soil surface) and a guide of the temperatures experienced at apex level in both of these conditions. **B**. Light intensity ranges used between the three institutes (RDA – South Korea: 100 µmol/m^2/s, University of Leeds – UK: 900 µmol/m^2/s, and University of Queensland – Australia: 1300 µmol/m^2/s,) during vernalisation. **C**. Light intensity ranges used between the three institutes (RDA – South Korea: 400 µmol/m^2/s, University of Leeds – UK: 450-500 µmol/m^2/s, and University of Queensland – Australia: 450-500 µmol/m^2/s) during speed breeding. An example of SGV–SB conditions using LED lighting. **D**. Representative image of plants in SGV conditions with **E**. showing the spectral measurement of light composition in SGV. **F.** Representative image of plants in SB conditions and **G**. showing the spectral measurement of light composition SB. Spectral measurements were taken using the RS-3500 spectroradiometer from spectral Evolution Inc. X-axis values are wavelengths in nanometres, and y-axis represents proportion (1 unit = 0.1 proportion).



Supplementary figure 4: Accelerated generation times also achieved for barley cultivars. **A**. Principal component analysis (PCA) of barley cultivars used in Figure 2. **B**. Spectral measurement of light composition during SGV for barley and **C**. Distribution and pairwise correlations for days to anthesis (DTA) for 60 diverse winter barley accessions evaluated using various vernalisation conditions presented in Figure 2A. The Pearson's correlation coefficient (r) displayed for each pairwise comparison for DTA were significant ($P < 0.05$).



Supplementary figure 5: Flowering distribution in populations formed from SGV-SB seeds. Three populations formed from spring x winter or winter x winter populations following SGV–SB; crosses 1 and 2 are spring x winter populations, and cross 3 is a winter x winter population.



Supplementary figure 6: Gene expression for *GIGANTEA* (red triangles), *PHYC* (green squares) and *CONSTANS-2* (blue circles) for cv. Hereward under **A**. SV and **B**. SGV conditions.

Supplementary Table 1. Days to heading (GS59) from germination of Korean cultivars and genetic resources under SB conditions. N = 4, s = spring growth habit, w =winter growth habit, - = unknown growth habit. Growth habits identified from field trials (Korea).

SB: 22 h light: 2 h dark, 22°C: 17°C

Group	Variety Name	Average	StDev
Group I : Speed breeding is effective	Baekkang (s)	40.5	2.1
	Hwanggeumal (s)	37.8	1.5
	Jokyung (s)	39.3	1.0
	Owolsomaek (s)	42.0	0.0
Group II : Speed breeding is non-effective	Alchanmil (w)	99.0	0.8
	Anbaekmil (w)	109.3	2.4
	Baekchal (w)	113.0	2.0
	Baekjoong (s)	95.8	8.3
	Chokwang (w)	116.5	2.6
	Cheongkyemil (w)	98.8	7.2
	Dabunmil (s)	114.8	4.5
	Dahongmil (w)	107.8	2.6
	Dajoongmil (s)	96.3	4.6
	Eunpamil (w)	92.3	3.8
	Geurumil (w)	104.8	3.6
	Gobunmil (w)	94.0	5.9
	Gosomil (s)	89.8	2.9
	Hanbaek (w)	100.0	5.5
	Hojoong (w)	85.0	1.4
	Jeokjoongmil (s)	96.8	2.2
	Jinpummil (w)	93.3	5.7
	Joah (s)	71.0	2.7
	Joeunmil (w)	106.5	5.1
	Jonong (w)	111.0	7.0
	Joongmo2008 (w)	103.8	2.6
	Jopummil (w)	83.0	1.4
	Milseoungmil (s)	85.7	0.6
	Namhaemil (w)	101.8	0.5

	Olmil (s)	86.0	0.0
	Saekeumgang (w)	112.8	6.5
	Saeolmil (s)	60.5	7.0
	Seodunmil (w)	96.3	3.9
	Sooan (w)	102.0	2.2
	Sugang (w)	93.5	5.7
	Tapdong (w)	107.3	3.6
	Urimil (w)	91.5	6.7
	Younbaek (w)	91.0	1.4
Group III (collected germplasm) : Speed breeding is non-effective	Icw77-0117-k-1ap-0ap-4ap-2 (-)	97.0	5.8
	Mk2538 (-)	NF	NF
	Mk2578 (-)	135.0	1.2
	NING MAI 50 (-)	86.8	9.7
	72957 (-)	126.5	7.5
	Swm11619-12ap-10ap-0ap (-)	NF	NF
	U00010192 (-)	NF	NF
	U00010257 (-)	NF	NF
	Yv 00-4 (-)	NF	NF
	Essai B (-)	NF	NF
	Norin 35 (-)	101.3	1.5
	BEZOSTAYA (-)	98.3	5.9
	Mk2381 (-)	NF	NF
	Norin16 (-)	NF	NF
	HIGOKU KOMUGI (w)	NF	NF
	Comache (w)	NF	NF
	RECITAL (w)	NF	NF
	Minhardi (w)	NF	NF

Supplementary Table 2: Heading date (GS59) in days to flowering from germination as an average n = 8 of winter growth habit varieties under each conditions, including VRN1 allelic type.

SB: 22 h light: 2 h dark, 22°C: 17°C

wRV–SB: 8 h light: 16 h dark 10°C into 22 h light: 2 h dark, 22°C: 17°C

SGV–RG: 22 h light: 2 h dark 10°C into 16 h light: 8 h dark, 22°C: 17°C

SGV–SB: 22 h light: 2 h dark 10°C into 22 h light: 2 h dark, 22°C: 17°C

Variety name	Condition	Average	StDev
Sturdy (*vrn-A1*, *vrn-B1* and *vrn-D1*)	SB	113.8	7.4
	wRV–SB	57.5	2.3
	SGV–RG	72.3	1.2
	SGV–SB	55.4	1.2
Nebred (*vrn-A1*, *vrn-B1* and *Vrn-D1*)	SB	106.6	3.7
	wRV–SB	62.9	1.4
	SGV–RG	76.1	0.8
	SGV–SB	60.9	1.7
Keumgang (*vrn-A1*, *vrn-B1* and *vrn-D1*)	SB	107.6	4.4
	wRV–SB	55.4	0.9
	SGV–RG	61.9	1.1
	SGV–SB	53.3	1.7

Supplementary Table 3. Heading date (GS59) in days to flowering from germination as an average n = 8 for four vernalisation treatments for the cv. Keumgang

Vernalisation conditions	Average	StDev
Seed vernalisation (T1)	59.8	1.0
Green vernalisation (T2)	59.0	0.5
Speed-green vernalisation with covering (T3)	57.6	3.8
Speed-green vernalisation without covering (T4)	53.1	0.8

Supplementary Table 4. Heading date (GS59) in days to flowering from germination of winter growth habit varieties according to vernalisation treatment temperature under SGV–SB conditions. N = 8

SGV–SB: 22 h light: 2 h dark for 4 weeks into 22 h light: 2 h dark, 22°C: 17°C

Variety name	Temperature(°C)	Average	StDev
Sturdy	22°C	113.8	7.4
	6°C	57.3	0.7
	8°C	56.4	0.9
	10°C	56.3	0.5
	12°C	63.5	3.0
Keumgang	22°C	107.6	4.4
	6°C	52.1	1.4
	8°C	53.1	2.0
	10°C	53.9	1.6
	12°C	58.5	0.8

Supplementary Table 5. Heading date (GS59) of winter growth habit varieties according to vernalisation treatment period, duration in weeks (w), under SGV–SB conditions. N = 8

SGV–SB: 22 h light: 2 h dark 10°C into 22 h light: 2 h dark 22°C: 17°C

Variety name	Duration (w)	Average	StDev
Sturdy	0	113.8	7.4
	1	83.9	3.4
	2	80.5	4.1
	3	54.5	2.1
	4	56.4	0.9
	5	61.4	0.5
	6	67.6	0.9
Keumgang	0	107.6	4.4
	1	84.5	2.5
	2	72.6	3.0
	3	50.5	1.5
	4	53.1	2.0
	5	58.1	0.8
	6	60.4	0.9

Supplementary Table 6A. Heading days (GS59) from germination of Korean cultivars and genetic resources under SGV–SB conditions and the *VRN1* allele regarding winter or spring habit on the A, B and D genome. Vrn = Spring/facilitative-habit (yellow and green), vrn = winter-habit (blue). NF = no flowering (after 150 days of growth), - data not known

Variety Name	Average	StDev	VRN1 alleles		
Baekkang	46.8	0.5			
Hwanggeumal	47.5	0.6			
Jokyung	50.5	0.6			
Owolsomaek	54.8	1.0			
Alchanmil	50.5	1.7	vrn-A1	vrn-B1	-
Anbaekmil	59.0	0.8	vrn-A1	vrn-B1	vrn-D1
Baekchal	55.0	0.0	vrn-A1	vrn-B1	vrn-D1
Baekjoong	50.5	0.6	vrn-A1	vrn-B1	-
Chokwang	52.3	1.0	vrn-A1	-	vrn-D1
Cheongkyemil	50.0	0.8	vrn-A1	vrn-B1	-
Dabunmil	58.3	3.3	vrn-A1	vrn-B1	vrn-D1
Dahongmil	53.8	1.0	vrn-A1	vrn-B1	vrn-D1
Dajoongmil	54.3	2.1	vrn-A1	-	vrn-D1
Eunpamil	49.0	0.8	Vrn-A1b	vrn-B1	vrn-D1
Geurumil	49.3	1.5	Vrn-A1b	-	-
Gobunmil	50.0	0.0	Vrn-A1b	vrn-B1	Vrn-D1
Gosomil	53.5	1.0	Vrn-A1b	-	vrn-D1
Hanbaek	53.5	1.0	vrn-A1	vrn-B1	vrn-D1
Hojoong	51.5	1.7	vrn-A1	vrn-B1	vrn-D1
Jeokjoongmil	50.8	1.5	vrn-A1	vrn-B1	-
Jinpummil	51.8	2.1	vrn-A1	vrn-B1	vrn-D1
Joah	49.3	0.5	Vrn-A1b	-	Vrn-D1
Joeunmil	51.3	3.2	vrn-A1	vrn-B1	vrn-D1
Jonong	53.0	0.0	vrn-A1	vrn-B1	vrn-D1
Joongmo2008	49.8	1.5	vrn-A1	vrn-B1	Vrn-D1
Jopummil	50.5	1.0	Vrn-A1a	vrn-B1	vrn-D1
Milseoungmil	48.0	0.0	vrn-A1	vrn-B1	-
Namhaemil	54.0	2.0	vrn-A1	vrn-B1	vrn-D1
Olmil	53.3	0.5	vrn-A1	vrn-B1	vrn-D1
Saekeumgang	56.0	0.0	vrn-A1	vrn-B1	vrn-D1
Saeolmil	48.8	1.5	vrn-A1	vrn-B1	Vrn-D1
Seodunmil	52.8	2.2	vrn-A1	vrn-B1	Vrn-D1
Sooan	54.0	0.0	vrn-A1	-	vrn-D1
Sugang	51.0	0.0	vrn-A1	vrn-B1	Vrn-D1
Tapdong	49.0	1.4	vrn-A1	vrn-B1	Vrn-D1
Urimil	50.5	1.3	vrn-A1	vrn-B1	Vrn-D1
Younbaek	53.5	1.0	vrn-A1	vrn-B1	Vrn-D1
Icw77-0117-k-1ap-0ap-4ap-2	50.8	3.9	vrn-A1	vrn-B1	vrn-D1
Mk2538	51.5	1.0	-	vrn-B1	Vrn-D1
Mk2578	80.0	1.4	vrn-A1	vrn-B1	vrn-D1
NING MAI 50	52.0	1.8	vrn-A1	vrn-B1	-
72957.0	60.5	1.9	Vrn-A1b	vrn-B1	vrn-D1
Swm11619-12ap-10ap-0ap	54.8	0.5	vrn-A1	vrn-B1	Vrn-D1
U00010192	54.3	1.9	vrn-A1	vrn-B1	Vrn-D1
U00010257	56.0	0.0	vrn-A1	Vrn-B1	Vrn-D1
Yv 00-4	73.3	1.0	vrn-A1	vrn-B1	vrn-D1
Essai B	64.3	2.1	vrn-A1	vrn-B1	vrn-D1
Norin 35	63.3	1.3	vrn-A1	vrn-B1	vrn-D1
BEZOSTAYA	54.8	0.5	vrn-A1	vrn-B1	vrn-D1
Mk2381	NF	NF	vrn-A1	vrn-B1	vrn-D1
Norin16	NF	NF	-	vrn-B1	vrn-D1
HIGOKU KOMUGI	NF	NF	vrn-A1	vrn-B1	vrn-D1
Comache	NF	NF	vrn-A1	vrn-B1	vrn-D1
RECITAL	NF	NF	vrn-A1	vrn-B1	vrn-D1
Minhardi	NF	NF	Vrn-A1b	vrn-B1	vrn-D1

Supplementary Table 6B. Summary of the types of germplasm according to VRN1 allelic classification, including flowering time range (DTH = days to heading) under SB and SGV_SB conditions. NF = no flowering.

Genotype				DTH in SB condition				DTH in SGV_SB condition			
VRN-A1	VRN-B1	VRN-D1	No. of cultivar	MIN	MAX	AVG	No. of cultivar NF	MIN	MAX	AVG	No. of cultivar NF
vrn-A1	vrn-B1	vrn-D1	24	85	135	105	6	51	80	57	4
vrn-A1	vrn-B1	Vrn-D1	12	39	107	83	2	49	56	52	0
vrn-A1	Vrn-B1	Vrn-D1	2	36	36	36	1	53	56	54	0
Vrn-A1a	vrn-B1	vrn-D1	2	42	83	63	0	51	55	53	0
Vrn-A1a	Vrn-B1	Vrn-D1	1	27	27	27	0	50	50	50	0
Vrn-A1b	vrn-B1	vrn-D1	3	92	127	109	1	49	61	55	1
Vrn-A1b	vrn-B1	Vrn-D1	4	31	94	51	0	47	51	49	0

Supplementary Table 7. Application of speed green vernalisation for wheat breeding program derived from a cross between Jokyung (spring) and Joongmo2008 (winter).

Generation	No. of plant (line)	Sowing	90% heading	Harvesting	Days/generation	Note
Cross	-	10/11/2018	-	25/05/2019	196	Field
F1	10	07/08/2019	-	01/11/2019	86	SGV–SB
F2	97	10/11/2019	06/01/2020	14/02/2020	96	SGV–SB
F3	167	24/02/2020	20/04/2020	20/05/2020	86	SGV–SB
F4	396	27/05/2020	17/07/2020	04/08/2020	69	SGV–SB
F5	264	19/08/2020	12/10/2020	02/11/2020	75	SGV–SB
F6	213	13/11/2020	23/04/2021	08/06/2021	207	Field

Supplementary Table 8. Primers used in RT-qPCR analysis.

IWGSC name	Gene name/function	Primer sequence	Direction	Source
TraesCS5A02G015600	ion channel/housekeeping	TCTAAATGTCCAGGAAGCTGTTA	sense	Borrill et al. (2016)
TraesCS5A02G015600	ion channel/housekeeping	CCTGTGGTGCCCAACTATT	anti-sense	
TraesCS5A02G391700	*VRN-A1*	GAACAAGATCAACCGGCAGGTGAC	sense	ADAPTAWHEAT project
TraesCS5A02G391700	*VRN-A1*	GGAGAAGATGATGAGGCCGACCTC	anti-sense	
TraesCS4B02G372700; TraesCS4D02G364500; TraesCS5A02G541300	*VRN2 (ZCCT1)*	GCCCACATCGTGCCATTTTACGGA	sense	This study
TraesCS4B02G372700; TraesCS4D02G364500; TraesCS5A02G541300	*VRN2 (ZCCT1)*	GCTCTCTCCTGCATTGTGGGATA	anti-sense	
TraesCS4D02G364400; TraesCS5A02G541200	*VRN2 (ZCCT2)*	CATCGTGCCATTCTGCGGG	sense	ADAPTAWHEAT project
TraesCS4D02G364400; TraesCS5A02G541200	*VRN2 (ZCCT2)*	CCCTGTACCTCATCACCTTCGCCT	anti-sense	
TraesCS7B02G013100	*FT-1B*	GTCGTTCGGGCAGGAG	sense	Shaw et al. (2012)
TraesCS7B02G013100	*FT-1B*	TGGAAGAGTACGAGCACGA	anti-sense	
TraesCS6A02G289400; TraesCS6B02G319500; TraesCS6D02G269500	*CONSTANS-2/Hd1*	CTTCCATCAGCAATGACATATC	sense	This study
TraesCS6A02G289400; TraesCS6B02G319500; TraesCS6D02G269500	*CONSTANS-2/Hd1*	GAAGTGAATGGCCTGAGAG	anti-sense	
TraesCS3A02G116300; TraesCS3B02G135400; TraesCS3D02G118200	*GIGANTEA*	TTCATTTCTTGCGTGCGATT	sense	This study
TraesCS3A02G116300; TraesCS3B02G135400; TraesCS3D02G118200	*GIGANTEA*	CTTCAACTCCTTCAGCATGC	anti-sense	
TraesCS5A02G391300; TraesCS5B02G396200; TraesCS5D02G401000	*PHYC*	TCTCAGGTATGCTTGCGAAT	sense	This study
TraesCS5A02G391300; TraesCS5B02G396200; TraesCS5D02G401000	*PHYC*	GTAACACAATGCTGCACCAT	anti-sense	

Supplementary Table 9. Cultivars used in this study

For the barley cultivars, Genotype ID links to the marker data available on Figshare (https://doi.org/10.6084/m9.figshare.19946045.v2).

Barley cultivars (*Hordeum vulgare*)				
Name	**ID (AUS)**	**Genotype ID**	**Origin**	**Growth type**
ARABI ABIAD	407642	UQ.AGG.182	SYRIA	Winter
ATLAS	400322	UQ.AGG.110	UNITED STATES	Winter
Balder	403550	UQ.AGG.163	SWEDEN	Winter
BELDI DWARF	412288	UQ.AGG.240	ALGERIA	Winter
BEY	495190	UQ.AGG.242	TURKEY	Winter
BLACK RUSSIAN	400451	UQ.AGG.741	FORMER SOVIET UNION	Winter
CIho 14259	406803	UQ.AGG.179	AFGHANISTAN	Winter
CIho 14776	419214	UQ.AGG.241	UNITED STATES	Winter
CIho 3835	409292	UQ.AGG.223	INDIA	Winter
CIho 4223	403102	UQ.AGG.129	CHILE	Winter
CIho 4223-2	403103	UQ.AGG.130	CHILE	Winter
CIho 6227	403275	UQ.AGG.141	TURKEY	Winter
CIho 6692	403393	UQ.AGG.150	TURKEY	Winter
ELSIS	400778	UQ.AGG.113	SOUTH AFRICA	Winter
ENTRESOLE	400785	UQ.AGG.114	BOLIVIA	Winter
FORRAJERA	403469	UQ.AGG.157	ARGENTINA	Winter
Guzluk	403511	UQ.AGG.742	TURKEY	Winter
H HOR 1018/59	400098	UQ.AGG.108	GREECE	Winter
HANACKY EXPORT	408718	UQ.AGG.743	CZECH REPUBLIC	Winter
HARLANJ.R.3904	403436	UQ.AGG.153	TURKEY	Winter
HARLANJ.R.456	403433	UQ.AGG.246	TURKEY	Winter
Hasaviurtovskij	408667	UQ.AGG.193	Russia	Winter
Heiligenblut Landgerste	408770	UQ.AGG.208	AUSTRIA	Winter
Hennersdorfer Silesia	408773	UQ.AGG.210	GERMANY	Winter
HOR 1447	408659	UQ.AGG.191	PORTUGAL	Winter
Horicky	408739	UQ.AGG.199	CZECH REPUBLIC	Winter
KAOSEIN	401224	UQ.AGG.244	CHINA	Winter
KENYA RESEARCH	410876	UQ.AGG.231	KENYA	Winter
KIKIN SHRAZE	408093	UQ.AGG.248	JAPAN	Winter
LISE	410882	UQ.AGG.232	NORWAY	Winter
LUBAS	401306	UQ.AGG.121	IRAQ	Winter
MUTANTE-66	403683	UQ.AGG.173	DENMARK	Winter
OCHSENHAUSENER RIA	408633	UQ.AGG.186	GERMANY	Winter
Orge No. 2	403172	UQ.AGG.134	MOROCCO	Winter
Orzo Vulcano 1921	403041	UQ.AGG.128	ITALY	Winter
PERESZTEGER OSTERR	408635	UQ.AGG.188	AUSTRIA	Winter
PI 125317	408948	UQ.AGG.215	AFGHANISTAN	Winter
PI 134260	403363	UQ.AGG.147	AFGHANISTAN	Winter
PI 138700	403381	UQ.AGG.148	IRAN	Winter
PI 138714	403385	UQ.AGG.149	IRAN	Winter
PI 168406	403437	UQ.AGG.247	TURKEY	Winter
PI 168421	403446	UQ.AGG.744	TURKEY	Winter
PI 170942	403458	UQ.AGG.155	TURKEY	Winter
PI 173521	403491	UQ.AGG.161	TURKEY	Winter
PI 183848	403603	UQ.AGG.168	TURKEY	Winter
PI 243615	408952	UQ.AGG.217	IRAN	Winter
PI 244829	402507	UQ.AGG.745	WESTERN ASIA	Winter
PI 370841	408956	UQ.AGG.219	AFGHANISTAN	Winter
PI 370841	409017	UQ.AGG.219	AFGHANISTAN	Winter
PI 47541	408947	UQ.AGG.214	IRAN	Winter
PI 68192	403129	UQ.AGG.132	AZERBAIJAN	Winter
PI 95176	408686	UQ.AGG.196	UKRAINE	Winter
PIONEER	401744	UQ.AGG.123	ENGLAND	Winter
PURPLE NUDUM	401798	UQ.AGG.245	PAKISTAN	Winter
QUANTUM	412284	UQ.AGG.239	AUSTRIA	Winter
Radosinsky Plnozrnny	408752	UQ.AGG.204	SLOVAKIA	Winter
Romi	403564	UQ.AGG.164	LEBANON	Winter
SZ5139B	403687	UQ.AGG.175	GERMANY	Winter
WELAM	410865	UQ.AGG.227	SWEDEN	Winter
WIR 13968	408604	UQ.AGG.185	Russia	Winter

Wheat cultivars (*Triticum aestivum*)			
Name	**ID (KOREA)**	**Origin**	**Growth type**
Buster		UK	Winter
Charger		UK	Winter
Claire		UK	Winter
Hereward		UK	Winter
Baekkang	332201	KOREAN	Spring
Hwanggeumal		KOREAN	Spring
Jokyung	213249	KOREAN	Spring
Owolsomaek	14380	KOREAN	Spring
Alchanmil	175574	KOREAN	Winter
Anbaekmil	213245	KOREAN	Winter
Baekchal	269494	KOREAN	Winter
Baekjoong	227093	KOREAN	Winter
Chokwang	116143	KOREAN	Winter
Cheongkyemil	172223	KOREAN	Winter
Dabunmil	227094	KOREAN	Winter
Dahongmil	175539	KOREAN	Winter
Dajoongmil	227076	KOREAN	Winter
Eunpamil	175521	KOREAN	Winter
Geurumil	159629	KOREAN	Winter
Gobunmil	214681	KOREAN	Winter
Gosomil	332393	KOREAN	Winter
Hanbaek	311644	KOREAN	Winter
Hojoong		KOREAN	Winter
Jeokjoongmil		KOREAN	Winter
Jinpummil	213099	KOREAN	Winter
Joah	275733	KOREAN	Winter
Joeunmil	213101	KOREAN	Winter
Jonong	215849	KOREAN	Winter
Joongmo2008		KOREAN	Winter
Jopummil	247761	KOREAN	Winter
Milseoungmil	246568	KOREAN	Winter
Namhaemil	15975	KOREAN	Winter
Olmil	15779	KOREAN	Winter
Saekeumgang	332202	KOREAN	Winter
Saeolmil	213244	KOREAN	Winter
Seodunmil	213098	KOREAN	Winter
Sooan	227074	KOREAN	Winter
Sugang	247762	KOREAN	Winter
Tapdong	159613	KOREAN	Winter
Urimil	175567	KOREAN	Winter
Younbaek	227127	KOREAN	Winter
Icw77-0117-k-1ap-0ap-4ap-2	166341		Winter
Mk2538	210444	MEXICO	Winter
Mk2578	210459	MEXICO	Winter
NING MAI 50	291176	CHINA	Winter
72957	166162		Winter
Swm11619-12ap-10ap-0ap	166405		Winter
U00010192	198245	MEXICO	Winter
U00010257	198174	MEXICO	Winter
Yv 00-4	293634	CHINA	Winter
Essai B	16061		Winter
Norin 35	16410	JAPAN	Winter
BEZOSTAYA	166199		Winter
Mk2381	210428	MEXICO	Winter
Norin16	12846	JAPAN	Winter
HIGOKU KOMUGI	13160	JAPAN	Winter
Comache	16016	MEXICO	Winter
RECITAL	166190		Winter
Minhardi	206063	USA	Winter
Sturdy	12021	USA	Winter
Keumgang	213100	KOREAN	Winter

Supplementary Table 10. Primers used to genotype *Vrn1* alleles.

IWGSC name	Allele	Primer name	Primer Sequence(5'-3')	Product size(bp)	Habit	Source
TraesCS5A02G391700	Vrn-A1a	VrnN_FP3	GTGTGTGTTTGTGGCGAGAG	926 (Vrn-A1a)	spring	Whittal et al. (2018)
	Vrn-A1b	VrnN_RP3	CGAAGGCGTATTGGGGAACA	633 (Vrn-A1b)	spring	
	vrn-A1	-	-	662 (vrn-A1)	winter	
TraesCS5B02G396600	Vrn-B1a	Intr/B/F	CAAGTGGAACGGTTAGGACA	709 (Vrn-B1a)	spring	Fu et al. (2005)
	Vrn-B1b	Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA	673 (Vrn-B1b)	spring	
	vrn-B1	Intr/B/F	CAAGTGGAACGGTTAGGACA	1149	winter	Fu et al. (2005)
		Intr1/B/R4	CAAATGAAAAGGAATGAGAGCA			
TraesCS5D02G401500	Vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	1671	spring	Fu et al. (2005)
		Intr1/D/R3	GGTCACTGGTGGTCTGTGC			
	vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC	997	winter	Fu et al. (2005)
		Intr1/D/R	AAATGAAAAGGAACGAGAGCG			

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