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Cite as: M. E. Jørgensen *et al.*, *Science* 10.1126/science.adx2022 (2025).

Postdomestication selection of MKK3 shaped seed dormancy and end-use traits in barley

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Anthropogenic selection of grain traits such as dormancy has shaped the developmental trajectories of crops. In cereals, shortening dormancy provides rapid and even post-harvest germination, but increases the risk of weather-induced pre-harvest sprouting (PHS) with yearly harvest losses beyond 1 billion USD. Our understanding of how, why, when and where cereal dormancy diversification arose is fragmentary. Here, we show in the founder crop barley (*Hordeum vulgare*) that dormancy is primarily regulated through a mosaic of locus haplotypes comprising copy-number variation and inherent kinase activity of *Mitogen-activated protein kinase kinase 3* (*MKK3*). We provide evidence supporting the historical selection of specific *MKK3* haplotypes that shape dormancy levels according to changing climatic pressures and outline a genetic framework for breeders to balance grain dormancy and PHS-avoidance.

The emergence of agriculture was driven by crop selection for enhanced overall performance, improved seed characteristics and adaption to a wide range of natural environments. Agriculture provided the foundation for modern human societies (*1*, *2*). Understanding how crop plants adapted to both anthropogenic and environmental demands over recent evolutionary time could therefore be central to developing long-term sustainable agricultural practices that cope with emerging global threats including our rapidly changing climate. However, the molecular mechanisms that underlie the successful adaptation of many key crop traits to different pressures remains poorly understood.

Dormancy in cereal grains (i.e., the suppression of germination in viable grains under environmental conditions normally favorable for germination) is one such trait. In wild cereal ancestors, adaptation to unpredictable or seasonal habitats has promoted variability in dormancy levels, a vital and evolutionary conserved bet-hedging strategy to ensure reproductive success of a population in case of catastrophic events (*3*). During domestication, anthropogenic selection shortened dormancy because grain with rapid and uniform germination optimised cultivation (*3*), while simplifying grain storage and subsequent processing. After spreading to temperate and sub-tropical areas, short periods of dormancy

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allowed for direct resowing of freshly harvested grain, enabling the production of two crops per year and significantly increasing annual yield (*4-8*). However, short dormancy duration may also promote the undesirable outcome of uncontrolled germination (sprouting) of mature grain before harvest in response to warm and moist weather, a phenomenon called pre-harvest sprouting (PHS) (Fig. 1A). PHS reduces the quality of mature grain and jeopardises its end-use value (*9*). Selection for short dormancy can thus have either beneficial or detrimental outcomes.

During the development of modern agriculture, alleles causing shorter grain dormancy frequently became genetically fixed within lineages to realize associated benefits. An unintended consequence is that environmentally triggered PHS continues to affect cereal crops in certain years and locations (*8, 10-16*), downgrading cereal grain quality and value, with yearly losses in the billion-dollar range (*17, 18*). With a predicted rise in atmospheric temperature alongside an increased frequency of extreme weather events (*19*), the incidence of PHS and associated crop loss will likely increase (*20*), intensifying the current food systems crisis (*21*). How to balance the avoidance of PHS with the benefits of reduced dormancy in cereals is therefore a high priority.

In the founder cereal crop barley, dormancy is considered a quantitative trait. *MKK3* has been identified as a major genetic component and exhibits diverse DNA sequence haplotypes reported to correlate with phenotypic variation (*11, 22*). By dissecting the molecular basis of *MKK3* haplotype variation in barley genome diversity panels combined with prebreeding and multi-year field trial validation we provide understanding of the directed evolution of cereal grain dormancy via environmental and cultural practices. We explore the origin and application of functionally diverse *MKK3* haplotypes, with the aim of implementing genome-enabled breeding for balanced and sustainable high-performance agriculture in changing environments (*23, 24*).

Grain dormancy and PHS in barley

In barley, a single amino acid exchange in MKK3 (MKK3^{T260}, i.e., Asparagine (N) to Threonine (T) at position 260), relative to the genomic reference HORVU.MOREX.PROJ.5HG00474740 (MKK3^{Ref}), lowers MKK3 kinase activity in vitro (*11*). This variant is associated with increased grain dormancy and reduced PHS in East Asian regions where wet harvests regularly promote PHS (*11*). In regions with a dry harvest climate such as the interior plains of North America, elite cultivars are associated with the opposite phenotype; low grain dormancy and high susceptibility to PHS in occasional wet harvest years. Genetic analyses (*13, 14, 25–27*) have shown that PHS susceptibility in this genetic material is correlated with an *MKK3* haplotype with a Glutamic Acid (E) to Glutamine (Q) exchange at position 165 (i.e., MKK3^{Q165}) (*26, 27*). To explore the generality of this observation we investigated *MKK3* sequence variation in the PHS-susceptible North American landmark cultivars (*cvs.*) Klages, Harrington and CDC Meredith and confirmed the presence of MKK3^{Q165}. However, the DNA sequencing signals appeared heterozygous for the mutation (fig. S1). As barley cultivars are homozygous inbred lines, we therefore checked *MKK3* for copy number variation (CNV), a common source of heterozygous genotyping calls, using droplet digital PCR (ddPCR). We found that *MKK3* was triplicated in all three accessions, with the MKK3^{Q165}-encoding variant present as two copies, thus explaining the heterozygous sequence signals (table S1). We then extended our CNV analysis to varieties currently recommended by the American Malting Barley Association (AMBA) (*26*) (table S1). We found both increased *MKK3* copy number and the MKK3^{Q165}-encoding variant in most AMBA lines and additionally CNV within batches of grain (Fig. 1B). This haplotype analysis indicates that the *MKK3* locus is more complex than previously reported and that heterogeneous *MKK3* haplotypes persist in elite breeding germplasm. Proposed genotype-phenotype associations for *MKK3* will therefore be inaccurate (*11, 25*), compromised by the genomic architecture of the *MKK3* locus and undermining breeding progress for PHS resilience (*27*).

The *MKK3* locus is complex in barley

To resolve these observations, we examined the *MKK3* locus in the barley pangenome (BPGv2) (*28*). Within the 76 pangenome sequences, we identified a total of 93 *MKK3* genes (defined as start to stop codon, including introns). Of these, 48 are distinct gene sequences. Restricting our analysis to coding sequences (CDS) revealed 24 CDS haplotypes encoding 13 unique MKK3 proteins (table S2). In 22 out of 23 wild barley (*Hordeum spontaneum*) accessions, we found a single full-length *MKK3* gene copy, compared with up to 5 tandemly duplicated or structurally re-arranged *MKK3* copies in domesticated barley as multiple sequence haplotypes (Fig. 1, C and D; table S2; and figs. S2 and S3A). Extending our analysis to a diverse collection of 365 ddPCR genotyped, 1,342 whole genome sequenced and 228 exome sequenced barley lines we found that wild barley accessions predominantly contain a single *MKK3*, while domesticated barleys show extensive CNV, with up to 15 copies including multi-copy mixed sequence haplotypes (Fig. 2A and tables S1, S3, and S4).

We next explored the functional impact of the observed CNV by analyzing transcript abundance variation in genotypes from the barley pan-transcriptome (Fig. 2B and table S5) (*29*). We found that first, European single-copy haplotypes (MKK3$^{R350+N383}$, [i.e., Glycine (G) to Arginine (N) at position 350 and Aspartic Acid (D) to Asparagine (N) at position 383], e.g., *cv.* RGT Planet) show a tendency for slightly higher *MKK3* transcript abundance compared to accessions

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associated with high dormancy, such as wild barleys (e.g., B1K-04-12 [FT11]), some landraces (e.g., HOR13942) and East Asian barleys (e.g., Akashinriki with MKK3^{T260}) (Fig. 2B and supplementary text 1.1). Secondly, *MKK3* copy number is positively correlated with transcript abundance across all five tissues tested (Fig. 2C and fig. S3B). Thus, minor differences in transcript abundance are observed among genotypes containing a single *MKK3*, but fold changes are a direct consequence of CNV in domesticates.

We then examined the global MKK3 haplotype diversity in 1,071 geo-referenced domesticated barley accessions (Fig. 3A and table S3), revealing distinct geographic patterns of haplotype enrichment. The high-dormancy variant MKK3^{T260} exhibited broader geographic distribution in regions with high precipitation and greater haplotype complexity than previously reported (Fig. 3A) (*11*, *30*). MKK3^{T260} was identified in combination with additional variants within both single- and multi-copy haplotypes (table S3). This suggests multiple independent evolutionary events, which potentially occurred as secondary adaptations to restore dormancy in domesticated barley as a consequence of range expansion. In contrast, the MKK3^{Q165} (low dormancy) multi-copy variant is predominantly found across Northern Europe and North America in 83 out of 1,707 accessions, but also in landraces with a single *MKK3* (HOR 10775 in CORE1000; HOR 10892 in BPGv2) (*28*) (Fig. 3A and table S3). A median-joining haplotype network analysis in the 76 pangenome sequence assemblies (BPGv2; *28*) indicates that MKK3^{Q165} arose independently as single- and multi-copy locus haplotypes (fig. S4A), suggesting that this variant causes a desired trait. The widely distributed MKK3^{Ref} is common across Europe, Asia and the Americas, whereas 41/43 MKK3^{V79} (i.e., Alanine (A) to Valine (V) at position 79) haplotypes are found in Ethiopian landraces. MKK3^{N383} and MKK3^{R350} variants are more common in Europe (Fig. 3A). The MKK3^{N383} variant is found in *Hordeum spontaneum* (wild barley) and in barleys secondary gene pool (*Hordeum bulbosum)* suggesting that the alternative allele MKK3^{D383} is a variant recently selected to increase dormancy (supplementary text 1.2) (*31*).

We next analyzed the link between specific MKK3 amino acid variants and inherent kinase activity. Using in vitro kinase assays (see Methods) we confirm that MKK3^{T260} decreases kinase activity and show that MKK3^{Q165} increases kinase activity (Fig. 3B and fig. S4B). Molecular modelling leads us to propose that Q165 promotes the evolutionary conserved protein kinase DFG amino acid motif toward an active state, increasing kinase activity (*32*) (Fig. 3C and supplementary text 1.3). Testing the other common amino acid exchanges MKK3^{N383} and MKK3^{V79} revealed that these show mild to strong increases in kinase activity, respectively (fig. S4C and supplementary text 1.3). We hypothesize that a combination of specific amino acid substitutions that alter MKK3 activity combined with CNV fine-tune overall MKK3 activity and consequently grain dormancy.

Variation at *MKK3* affects dormancy and PHS susceptibility

To assess the practical impact of different *MKK3* locus haplotypes on dormancy and PHS we conducted field trials over several seasons with half of the plots of each genotype harvested at maturity in dry conditions and the other half exposed to rain and/or spray irrigation to promote the initiation of PHS (Fig. 4A). Harvested grain material was analyzed using well-established tests from the barley malting industry; germination energy to measure grain dormancy and germination index to measure the speed of germination (see Methods). In 2023, we compared *cv.* RGT Planet (MKK3$^{R350+N383}$), *cv.* Morex (MKK3^{Ref}/MKK3^{Ref}) and *cv.* Harrington (MKK3^{Ref}/MKK3^{Q165}/MKK3^{Q165}) dry harvest samples, assaying germination energy and germination index at 2 and 10 weeks after harvest. Dormancy only became fully broken after 10 weeks for *cv.* RGT Planet, while *cvs.* Harrington and Morex, with increased *MKK3* copy number and with/without hyperactive MKK3^{Q165}, respectively, had little grain dormancy (Fig. 4B).

We then explored the impact of different *MKK3* locus haplotypes on germination energy and germination index in a common genetic background. We used marker-assisted breeding to assemble six near-isogenic lines (NIL1–NIL6) in *cv.* RGT Planet by introgressing various *MKK3* locus haplotypes from a range of donors (table S6). We included a FIND-IT premature stop codon variant (MKK3^{*270}, i.e., Tryptophan (W) to stop at position 270) in the RGT Planet genetic background (*33*). In our PHS field trials, a genotype prone to PHS will show a reduction in germination energy and/or germination index in the rain-exposed samples, because re-germination of a pre-sprouted grain is suspended or decelerated, respectively. We analyzed germination characteristics of both dry and wet-harvested grain in 2023 and 2024. Assessing the impact of *MKK3* locus haplotype on germination energy and germination index at three weeks post dry harvest (only 2024 season; fig. S5, A and B), where dormant cultivars (e.g., *cv.* RGT Planet) still possessed significant dormancy, showed that isolines containing multiple *MKK3* copies or MKK3^{Q165} in single- or multi-copy had no dormancy. The *cv.* RGT Planet knockout variant MKK3^{*270} was fully dormant, supporting *MKK3* as the major dormancy controller.

Next, we compared germination energy and germination index in the PHS-inducing wet-harvest grain at 10 weeks (i.e., after dormancy is broken: Fig. 4C and fig. S5C) in each NIL to the dry-harvest samples (2023 and 2024 season). We anticipated a drop in germination energy and germination index after PHS induction. In the wet-harvested samples from 2023, we observed germination energy that was (*i*) unchanged or

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mildly reduced (i.e., up to 5% in RGT Planet and NIL1 (MKK3^{T260}, respectively), (*ii*) strongly reduced (i.e., up to 25% in NIL2 (MKK3^{Ref}/MKK3^{Ref}) and NIL3 (MKK3^{Ref}/MKK3^{Ref}/MKK3^{Ref}/MKK3^{Ref})) and (*iii*) very strongly reduced (i.e., up to 60% in NIL4 (MKK3^{Q165}) and NILs 5 and 6 (MKK3^{Ref}/MKK3^{Q165}/MKK3^{Q165})) (Fig. 4D). We found a similar pattern for germination index, with the noticeable difference that multi-copy haplotypes (NIL2/3) are as strongly affected as the NILs with the MKK3^{Q165} variant in single- and multi-copy haplotypes (NIL 4/5/6) (Fig. 4E). In the 2024 season, where PHS inducing conditions were suboptimal (fig. S5, D and E) we saw no significant difference between the dry and PHS harvest germination for the multi-copy haplotypes (NIL2/3; fig. S5, F and G). In summary, different germination energy and germination index responses of isolines under both dry and wet harvest conditions confirmed our hypothesis that dormancy is fine-tuned by different *MKK3* haplotypes.

MKK3 adaptation to environment, agricultural practice and culture

To understand if haplotype-dependent fine-tuning of dormancy arose through association with environmental, agricultural and/or cultural habits we compared selection pressure between domesticated and wild barleys at the variant level. This showed an increase in the frequency of non-synonymous variants in domesticated barley. In contrast, synonymous variant frequencies were not statistically different between wild and domesticated barley (supplementary text 1.4). This increased selection of *MKK3* variants in domesticated barley was supported by a median-joining haplotype network analysis of georeferenced wild and domesticated accessions that showed an expansion of haplotype diversity in domesticated barley, whereas wild barley accessions had minimal and dispersed haplotypes (data S1, table S7, and supplementary text 1.4).

We then explored the demographic distribution of selected barley *MKK3* haplotypes at a global scale. Genotypes with single-copy MKK3^{T260} (i.e., unaffected by PHS-inducing conditions) were found in regions exposed to very high PHS risk due to the co-occurrence of harvest and monsoon weather in the region (Figs. 3A and 4, C to E) (*11*). In contrast, traditional agricultural practices in certain regions of the world (e.g., the highlands of Pamir (Tajikistan, Kyrgyzstan, Pakistan, Afghanistan), the Tibetan plateau (China, Bhutan, Nepal) and Ethiopia favor barleys with no dormancy (*4–6*, *34*, *35*). Genotypes with the hyperactive Ethiopian MKK3^{V79} variant (supplementary text 1.5) and low dormancy match the suitability for double-cropping which increases annual yield and avoids long-term storage and associated pest problems (*4–6*, *34*). Tibetan (hull-less) barleys contain many *MKK3* copies and associated lower dormancy in years with high PHS risk (Fig. 2A; fig. S5, C, F, and G; and tables S1 and S3). In this region, harvest frequently occurs before the grain is fully matured and air-dried prior to processing (roasting and grinding) for human consumption and storage over winter (*35*). Our field experiments showed that multi-copy haplotypes (e.g., NIL2/3) have little grain dormancy, but that germination index was less severely affected by PHS induction (Fig. 4, C to E, and fig. S5, C, F, and G). We suggest that a key attribute of the stored seed is the maintenance of vigour. After planting, seeds are then primed to cope with harsh short-season environments allowing most grains to germinate and establish seedlings.

A hyperactive MKK3 originated in Nordic agriculture

We found multi-copy and hyperactive *MKK3* locus haplotypes in barleys grown in temperate and sub-polar regions (e.g., North America, Scandinavia and the North Atlantic Islands). Re-tracing pedigrees suggested that the MKK3^{Ref}/MKK3^{Q165}/MKK3^{Q165} haplotype that persists in PHS-susceptible North American malting barleys (table S1) was derived from the Norwegian *cv*. Domen (*36*) released in the 1950s. Using ddPCR-based genotyping, we traced this Domen haplotype to far older landraces (e.g., Jotun, Asplund, Maskin, Björneby) originating from across Scandinavia, Scotland, the Faroe Islands, Iceland and Russia (Fig. 3A and table S1), where multi-copy *MKK3* locus haplotypes with at least one MKK3^{Q165} variant are found. Extending our haplotype analysis across the *MKK3* locus to a selection of Northern European barley landraces and modern elite varieties using 50K-SNP-array data (*37*) (table S1) revealed that the Scandinavian landraces carrying MKK3^{Q165} cluster together with Bere barleys, the oldest known barley landraces in northern Europe (data S2). Bere is still cultivated on the Northern and Western Isles of Scotland where it was introduced more than 5,000 years ago (*38*). ddPCR analyses of 28 different Bere accessions (*39*) revealed the presence of MKK3^{Q165} in all lines alongside variation in copy number (1-4 copies) (Fig. 2A and table S1). Thus, Bere may well be the ancestral Northern European donor of MKK3^{Q165}.

In these wet sub-polar regions, barley was selected for quality characteristics that emerge after controlled germination (malting), which is key to the production of alcoholic beverages (beer). Prior quantitative genetic analyses have mapped components of malting quality to the same region as PHS (*16*, *25*). Through micro-malting and analysis of *MKK3* NILs 1-6 we found that haplotypes containing at least one hyperactive MKK3^{Q165} (i.e., NILs 4/5/6) have elevated malt quality characteristics including high α-amylase, free limit dextrinase and (1,3;1,4)-β-glucanase enzyme activities (fig. S6, A to C). These qualities are in particularly high demand for brewing beer from a mix of barley malt and other starchy grains (*40*). However, these rapid germination characteristics

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come with the trade-off of increased susceptibility to PHS (Fig. 4F and fig. S6, D and E). Records from the 18th century describe extinct Norwegian landraces of the 'Thorebygg' type as pre-17th century barleys with high sensitivity to moisture during the autumn harvest period (i.e., PHS) (*41*). These Norwegian landraces were prized for their superior quality characteristics needed for the brewing of traditional farmhouse ales, which used barley malt along with other more widely available starchy cereal grains such as oats, that are normally less suitable for brewing (*42*). If they are derived from Scottish Bere barleys it is reasonable to assume that over 1,000 years ago, early Viking farmers would have valued barley with MKK3^{Q165} haplotypes for brewing so highly that they carried grains with them on their travels, developing specific agricultural practices to avoid PHS, such as pre-mature harvest followed by smoke-drying (*42*). They might have been largely responsible for its primary distribution (i.e., within Northern Europe), while a secondary distribution may have been driven by modern breeding programmes, first successfully to Canada and then to Australia where MKK3^{Q165} failed due to its sensitivity to local climatic conditions. Together, this illustrates how a trait with such a strong end use value can be sought and persevere despite a strong risk for crop loss.

Discussion

The rise of agriculture initiated arguably the greatest single step development in the history of humans. It required that newly domesticated crops adapted to diverse and changing environmental demands. We provide evidence that human intervention shaped one such trait in barley, post-harvest grain dormancy, through the structural and functional landscape of *MKK3* via three distinct molecular mechanisms: 1) variation in copy number that directly influences transcript abundance, 2) selection and maintenance of *MKK3* amino acid variants with altered kinase activity and 3) distinct combinations of these individual mechanisms. Some of the resulting haplotypes have gained prominence in diverse regional environments as they have characteristics that increase crop productivity and value. The *MKK3* haplotype complexity found here showcases a hidden challenge in the traditional cross-breeding of crops that requires applied pangenomics, in this case to avoid high costs from grain loss and quality downgrading.

This study raises an important evolutionary question; is the immense complexity and dynamic nature of the barley *MKK3* locus unique? Between species, MKK3 is a key dormancy regulator across multiple domesticated cereals including rice (*12*) and wheat (*10*) that will have responded to similar selection pressures. Within species, the barley pangenome identified more than 150 structurally complex loci (*28*), prompting us to ask how many post-domestication trait-associated loci will exhibit a similarly complex functional haplotype diversity? Functional annotation of these complex loci indicates their involvement in a range of high-value traits including disease and herbivory resistance, plant architecture and grain quality (*28*). We are tempted to speculate that their post-domestication evolution may similarly reflect a consequence of environmental and anthropogenic selection for specific high-value traits.

A key insight from our study is that the balance between environmental resilience and end use performance of a crop is a delicate one. We show that joint exploration of exotic crop germplasm at the genomic level combined with appropriate phenotypic analyses can provide valuable clues about genetic variants that, when introduced into modern genotypes, may provide a balanced and sustainable solution for the challenges of today.

REFERENCES AND NOTES

1. M. D. Purugganan, What is domestication? *Trends Ecol. Evol.* **37**, 663–671 (2022). doi:10.1016/j.tree.2022.04.006 Medline
2. J. Diamond, Evolution, consequences and future of plant and animal domestication. *Nature* **418**, 700–707 (2002). doi:10.1038/nature01019 Medline
3. D. Q. Fuller, R. Allaby, "Seed dispersal and crop domestication: Shattering, germination and seasonality in evolution under cultivation" in *Annual Plant Reviews Volume 38: Fruit Development and Seed Dispersal*, L. Østergaard, Ed. (Wiley, 2009), pp. 238–295.
4. A. Hussain, F. M. Qamar, L. Adhikari, A. I. Hunzai, A. U. Rehman, K. Bano, Climate change, mountain food systems, and emerging opportunities: A study from the Hindu Kush Karakoram Pamir Landscape, Pakistan. *Sustainability (Basel)* **13**, 3057 (2021). doi:10.3390/su13063057
5. D. L. Lister, H. Jones, H. R. Oliveira, C. A. Petrie, X. Liu, J. Cockram, C. J. Kneale, O. Kovaleva, M. K. Jones, Barley heads east: Genetic analyses reveal routes of spread through diverse Eurasian landscapes. *PLOS ONE* **13**, e0196652 (2018). doi:10.1371/journal.pone.0196652 Medline
6. H. Gebre, F. Alemayehu, "Indigenous barley germplasm in the ethiopian breeding programme: in *Plant Genetic Resources of Ethiopia*, J. M. M. Engels, J. G. Hawkes, M. Worede, Eds. (Cambridge Univ. Press, 1991), pp. 303–314.
7. T. T. Chang, The impact of rice on human civilization and population expansion. *Interdiscip. Sci. Rev.* **12**, 63–69 (1987). doi:10.1179/isr.1987.12.1.63
8. G. M. Paulsen, A. S. Auld, "Preharvest sprouting of cereals" in *Handbook of Seed Physiology Applications to Agriculture* (Haworth Press, 2004), pp. 199–219.
9. G. Kaur, P. K. Toora, P. A. Tuan, C. A. McCartney, M. S. Izydorczyk, A. Badea, B. T. Ayele, Genome-wide association and targeted transcriptomic analyses reveal loci and candidate genes regulating preharvest sprouting in barley. *Theor. Appl. Genet.* **136**, 202 (2023). doi:10.1007/s00122-023-04449-0 Medline
10. A. Torada, M. Koike, T. Ogawa, Y. Takenouchi, K. Tadamura, J. Wu, T. Matsumoto, K. Kawaura, Y. Ogihara, A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase kinase. *Curr. Biol.* **26**, 782–787 (2016). doi:10.1016/j.cub.2016.01.063 Medline
11. S. Nakamura, M. Pourkheirandish, H. Morishige, Y. Kubo, M. Nakamura, K. Ichimura, S. Seo, H. Kanamori, J. Wu, T. Ando, G. Hensel, M. Sameri, N. Stein, K. Sato, T. Matsumoto, M. Yano, T. Komatsuda, Mitogen-activated protein kinase kinase 3 regulates seed dormancy in barley. *Curr. Biol.* **26**, 775–781 (2016). doi:10.1016/j.cub.2016.01.024 Medline
12. X. Mao, X. Zheng, B. Sun, L. Jiang, J. Zhang, S. Lyu, H. Yu, P. Chen, W. Chen, Z. Fan, C. Li, Q. Liu, MKK3 Cascade Regulates Seed Dormancy Through a Negative Feedback Loop Modulating ABA Signal in Rice. *Rice (N. Y.)* **17**, 2 (2024). doi:10.1186/s12284-023-00679-4 Medline
13. C. D. Li, A. Tarr, R. C. M. Lance, S. Harasymow, J. Uhlmann, S. Westcot, K. J. Young, C. R. Grime, M. Cakir, S. Broughton, R. Appels, A major QTL controlling seed dormancy and pre-harvest sprouting/grain α-amylase in two-rowed barley (Hordeum vulgare L.). *Aust. J. Agric. Res.* **54**, 1303–1313 (2003). doi:10.1071/AR02210

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14. A. R. Barr, A. Karakousis, R. C. M. Lance, S. J. Logue, S. Manning, K. J. Chalmers, J. M. Kretschmer, W. J. R. Boyd, H. M. Collins, S. Roumeliotis, S. J. Coventry, D. B. Moody, B. J. Read, D. Poulsen, C. D. Li, G. J. Platz, P. A. Inkerman, J. F. Panozzo, B. R. Cullis, A. B. Smith, P. Lim, P. Langridge, Mapping and QTL analysis of the barley population Chebec× Harrington. *Aust. J. Agric. Res.* **54**, 1125–1130 (2003). doi:10.1071/AR02215
15. A. L. Hills, B. H. Paynter, S. Harasymow, A. Tarr, "New malting barley varieties less susceptible to pre-harvest sprouting than Hamelin and Flagship," paper presented at the 14th Australian Barley Technical Symposium, Perth, Western Australia, 13–16 September 2009.
16. M. E. Looseley, L. Ramsay, H. Bull, J. S. Swanston, P. D. Shaw, M. Macaulay, A. Booth, J. R. Russell, R. Waugh, W. T. B. Thomas; IMPROMALT Consortium, Association mapping of malting quality traits in UK spring and winter barley cultivar collections. *Theor. Appl. Genet.* **133**, 2567–2582 (2020). doi:10.1007/s00122-020-03618-9 Medline
17. J. Bucheli, M. Visse-Mansiaux, J. Herrera, L. L. Häner, J. Tack, R. Finger, Precipitation causes quality losses of large economic relevance in wheat production. *Q Open* **4**, qoae008 (2024). doi:10.1093/qopen/qoae008
18. M. Black, J. D. Bewley, P. Halmer, *The Encyclopaedia of Seeds: Science, Technology and Uses* (CABI, 2006).
19. B. Clarke, F. Otto, R. Stuart-Smith, L. Harrington, Extreme weather impacts of climate change: An attribution perspective. *Environ. Res. Climate* **1**, 012001 (2022). doi:10.1088/2752-5295/ac6e7d
20. J. S. Lee, D. Chebotarov, K. L. McNally, V. Pede, T. D. Setiyono, R. Raquid, W. J. Hyun, J. U. Jeung, A. Kohli, Y. Mo, Novel sources of pre-harvest sprouting resistance for japonica rice improvement. *Plants* **10**, 1709 (2021). doi:10.3390/plants10081709 Medline
21. Food loss and waste. *Nat. Food* **5**, 639 (2024). doi:10.1038/s43016-024-01041-7 Medline
22. S. Nakamura, Grain dormancy genes responsible for preventing pre-harvest sprouting in barley and wheat. *Breed. Sci.* **68**, 295–304 (2018). doi:10.1270/jsbbs.17138 Medline
23. Z. Tian, J. W. Wang, J. Li, B. Han, Designing future crops: Challenges and strategies for sustainable agriculture. *Plant J.* **105**, 1165–1178 (2021). doi:10.1111/tpj.15107 Medline
24. M. Mascher, M. Jayakodi, H. Shim, N. Stein, Promises and challenges of crop translational genomics. *Nature* **636**, 585–593 (2024). doi:10.1038/s41586-024-07713-5 Medline
25. T. E. Rooney, D. W. Sweeney, K. H. Kunze, M. E. Sorrells, J. G. Walling, Malting quality and preharvest sprouting traits are genetically correlated in spring malting barley. *Theor. Appl. Genet.* **136**, 59 (2023). doi:10.1007/s00122-023-04257-6 Medline
26. J. M. Vetch, J. G. Walling, J. Sherman, J. M. Martin, M. J. Giroux, Mutations in the HvMKK3 and HvAlaAT1 genes affect barley preharvest sprouting and after-ripened seed dormancy. *Crop Sci.* **60**, 1897–1906 (2020). doi:10.1002/csc2.20178
27. D. W. Sweeney, T. E. Rooney, J. G. Walling, M. E. Sorrells, Interactions of the barley SD1 and SD2 seed dormancy loci influence preharvest sprouting, seed dormancy, and malting quality. *Crop Sci.* **62**, 120–138 (2022). doi:10.1002/csc2.20641
28. M. Jayakodi, Q. Lu, H. Pidon, M. T. Rabanus-Wallace, M. Bayer, T. Lux, Y. Guo, B. Jaegle, A. Badea, W. Bekele, G. S. Brar, K. Braune, B. Bunk, K. J. Chalmers, B. Chapman, M. E. Jørgensen, J.-W. Feng, M. Feser, A. Fiebig, H. Gundlach, W. Guo, G. Haberer, M. Hansson, A. Himmelbach, I. Hoffie, R. E. Hoffie, H. Hu, S. Isobe, P. König, S. M. Kale, N. Kamal, G. Keeble-Gagnère, B. Keller, M. Knauft, R. Koppolu, S. G. Krattinger, J. Kumlehn, P. Langridge, C. Li, M. P. Marone, A. Maurer, K. F. X. Mayer, M. Melzer, G. J. Muehlbauer, E. Murozuka, S. Padmarasu, D. Perovic, K. Pillen, P. A. Pin, C. J. Pozniak, L. Ramsay, P. R. Pedas, T. Rutten, S. Sakuma, K. Sato, D. Schüler, T. Schmutzer, U. Scholz, M. Schreiber, K. Shirasawa, C. Simpson, B. Skadhauge, M. Spannagl, B. J. Steffenson, H. C. Thomsen, J. F. Tibbits, M. T. S. Nielsen, C. Trautewig, D. Vequaud, C. Voss, P. Wang, R. Waugh, S. Westcott, M. W. Rasmussen, R. Zhang, X.-Q. Zhang, T. Wicker, C. Dockter, M. Mascher, N. Stein, Structural variation in the pangenome of wild and domesticated barley. *Nature* **636**, 654–662 (2024). doi:10.1038/s41586-024-08187-1 Medline
29. W. Guo, M. Schreiber, V. B. Marosi, P. Bagnaresi, M. E. Jørgensen, K. B. Braune, K. Chalmers, B. Chapman, V. Dang, C. Dockter, A. Fiebig, G. B. Fincher, A. Fricano, J. Fuller, A. Haaning, G. Haberer, A. Himmelbach, M. Jayakodi, Y. Jia, N. Kamal, P. Langridge, C. Li, Q. Lu, T. Lux, M. Mascher, K. F. X. Mayer, N. McCallum, L. Milne, G. J. Muehlbauer, M. T. S. Nielsen, S. Padmarasu, P. R. Pedas, K. Pillen, C. Pozniak, M. W. Rasmussen, K. Sato, T. Schmutzer, U. Scholz, D. Schüler, H. Šimková, B. Skadhauge, N. Stein, N. W. Thomsen, C. Voss, P. Wang, R. Wonneberger, X.-Q. Zhang, G. Zhang, L. Cattivelli, M. Spannagl, M. Bayer, C. Simpson, R. Zhang, R. Waugh, A barley pan-transcriptome reveals layers of genotype-dependent transcriptional complexity. *Nat. Genet.* **57**, 441–450 (2025). doi:10.1038/s41588-024-02069-y Medline
30. S. E. Fick, R. J. Hijmans, WorldClim 2: New 1km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **37**, 4302–4315 (2017). doi:10.1002/joc.5086
31. J. W. Feng, H. Pidon, M. Cuacos, T. Lux, A. Himmelbach, R. Haghi, J. Fuchs, G. Haberer, Y.-T. Kuo, Y. Guo, M. Jayakodi, H. Toegelová, D. Harpke, M. Knauft, A. Fiebig, M. Maruschewski, M. Ronen, A. Sharon, H. Šimková, K. F. X. Mayer, M. Spannagl, J. Kumlehn, S. Heckmann, A. Houben, F. R. Blattner, N. Stein, M. Mascher, A haplotype-resolved pangenome of the barley wild relative Hordeum bulbosum. *Nature* **645**, 429–438 (2025). doi:10.1038/s41586-025-09270-x Medline
32. C. Arter, L. Trask, S. Ward, S. Yeoh, R. Bayliss, Structural features of the protein kinase domain and targeted binding by small-molecule inhibitors. *J. Biol. Chem.* **298**, 102247 (2022). doi:10.1016/j.jbc.2022.102247 Medline
33. S. Knudsen, T. Wendt, C. Dockter, H. C. Thomsen, M. Rasmussen, M. Egevang Jørgensen, Q. Lu, C. Voss, E. Murozuka, J. T. Østerberg, J. Harholt, I. Braumann, J. A. Cuesta-Seijo, S. M. Kale, S. Bodevin, L. Tang Petersen, M. Carciofi, P. R. Pedas, J. Opstrup Husum, M. T. S. Nielsen, K. Nielsen, M. K. Jensen, L. A. Møller, Z. Gojkovic, A. Striebeck, K. Lengeler, R. T. Fennessy, M. Katz, R. Garcia Sanchez, N. Solodovnikova, J. Förster, O. Olsen, B. L. Møller, G. B. Fincher, B. Skadhauge, FIND-IT: Accelerated trait development for a green evolution. *Sci. Adv.* **8**, eabq2266 (2022). doi:10.1126/sciadv.abq2266 Medline
34. K. Hori, K. Sato, K. Takeda, Detection of seed dormancy QTL in multiple mapping populations derived from crosses involving novel barley germplasm. *Theor. Appl. Genet.* **115**, 869–876 (2007). doi:10.1007/s00122-007-0620-3 Medline
35. L. Tang, H. Lu, J. Song, S. Wangdue, X. Chen, Z. Zhang, X. Liu, N. Boivin, R. N. Spengler III, The transition to a barley-dominant cultivation system in Tibet: First millennium BC archaeobotanical evidence from Bangga. *J. Anthropol. Archaeol.* **61**, 101242 (2021). doi:10.1016/j.jaa.2020.101242
36. B. L. Harvey, B. G. Rossnagel, R. P. Muderewich, "Sprouting resistance in barley" in *Third International Symposium on Preharvest Sprouting in Cereals*, J. E. Kruger, D. E. LaBerge, Eds. (CRC Press, 2019), pp. 239–243.
37. S. B. Schmidt, T. S. George, L. K. Brown, A. Booth, J. Wishart, P. E. Hedley, P. Martin, J. Russell, S. Husted, Ancient barley landraces adapted to marginal soils demonstrate exceptional tolerance to manganese limitation. *Ann. Bot.* **123**, 831–843 (2019). doi:10.1093/aob/mcy215 Medline
38. R. R. Bishop, D. R. Gröcke, I. Ralston, D. Clarke, D. H. Lee, A. Shepherd, A. S. Thomas, P. A. Rowley-Conwy, M. J. Church, Scotland's first farmers: New insights into early farming practices in North-west Europe. *Antiquity* **96**, 1087–1104 (2022). doi:10.15184/aqy.2022.107
39. P. Martin, J. Russell, J. Wishart, L. K. Brown, M. Wallace, P. P. M. Iannetta, T. S. George, Back to the future: Using ancient Bere barley landraces for a sustainable future. *Plants People Planet* **7**, 546–561 (2023). doi:10.1002/ppp3.10432
40. G. Oliver, *The Oxford Companion to Beer* (Oxford Univ. Press, 2012).
41. H. O. Bråtå, The Naked Barley Thorebygg and Norwegian Farmer's Ale. *Glob. Food Hist.* **8**, 85–105 (2022). doi:10.1080/20549547.2022.2045168
42. L. M. Garshol, *Historical brewing techniques: The lost art of farmhouse brewing* (Brewers Publications, 2020).
43. K. Katoh, H. Toh, Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* **9**, 286–298 (2008). doi:10.1093/bib/bbn013 Medline
44. R Core Team, "R: A language and environment for statistical computing" (R Foundation for Statistical Computing, 2022).
45. M. Schreiber, R. Wonneberger, A. Haaning, "Data record for the genomic resources of cultivated European two-rowed spring barley genotypes" (Leibniz Institute of Plant Genetics and Crop Plant Research, 2023); https://doi.org/10.5447/IPK/2023/15.
46. M. Schreiber, R. Wonneberger, A. M. Haaning, M. Coulter, J. Russell, A.

Downloaded from https://www.science.org at Helmholtz Zentrum München - Zentralbibliothek on November 07, 2025

Himmelbach, A. Fiebig, G. J. Muehlbauer, N. Stein, R. Waugh, Genomic resources for a historical collection of cultivated two-row European spring barley genotypes. *Sci. Data* **11**, 66 (2024). doi:10.1038/s41597-023-02850-4 Medline
47. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410 (1990). doi:10.1016/S0022-2836(05)80360-2 Medline
48. M. Mascher, T. Wicker, J. Jenkins, C. Plott, T. Lux, C. S. Koh, J. Ens, H. Gundlach, L. B. Boston, Z. Tulpová, S. Holden, I. Hernández-Pinzón, U. Scholz, K. F. X. Mayer, M. Spannagl, C. J. Pozniak, A. G. Sharpe, H. Šimková, M. J. Moscou, J. Grimwood, J. Schmutz, N. Stein, Long-read sequence assembly: A technical evaluation in barley. *Plant Cell* **33**, 1888–1906 (2021). doi:10.1093/plcell/koab077 Medline
49. H. Li, Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics* **34**, 3094–3100 (2018). Medline
50. J. W. Leigh, D. Bryant, POPART: Full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116 (2015). doi:10.1111/2041-210X.12410
51. J. Russell, M. Mascher, I. K. Dawson, S. Kyriakidis, C. Calixto, F. Freund, M. Bayer, I. Milne, T. Marshall-Griffiths, S. Heinen, A. Hofstad, R. Sharma, A. Himmelbach, M. Knauft, M. van Zonneveld, J. W. S. Brown, K. Schmid, B. Kilian, G. J. Muehlbauer, N. Stein, R. Waugh, Exome sequencing of geographically diverse barley landraces and wild relatives gives insights into environmental adaptation. *Nat. Genet.* **48**, 1024–1030 (2016). doi:10.1038/ng.3612 Medline
52. G. Marçais, C. Kingsford, A fast, lock-free approach for efficient parallel counting of occurrences of *k*-mers. *Bioinformatics* **27**, 764–770 (2011). doi:10.1093/bioinformatics/btr011 Medline
53. M. Jayakodi, S. Padmarasu, G. Haberer, V. S. Bonthala, H. Gundlach, C. Monat, T. Lux, N. Kamal, D. Lang, A. Himmelbach, J. Ens, X.-Q. Zhang, T. T. Angessa, G. Zhou, C. Tan, C. Hill, P. Wang, M. Schreiber, L. B. Boston, C. Plott, J. Jenkins, Y. Guo, A. Fiebig, H. Budak, D. Xu, J. Zhang, C. Wang, J. Grimwood, J. Schmutz, G. Guo, G. Zhang, K. Mochida, T. Hirayama, K. Sato, K. J. Chalmers, P. Langridge, R. Waugh, C. J. Pozniak, U. Scholz, K. F. X. Mayer, M. Spannagl, C. Li, M. Mascher, N. Stein, The barley pan-genome reveals the hidden legacy of mutation breeding. *Nature* **588**, 284–289 (2020). doi:10.1038/s41586-020-2947-8 Medline
54. B. Bushnell, J. Rood, E. Singer, BBMerge - Accurate paired shotgun read merging via overlap. *PLOS ONE* **12**, e0185056 (2017). doi:10.1371/journal.pone.0185056 Medline
55. M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* **17**, 10–12 (2011). doi:10.14806/ej.17.1.200
56. M. Kokot, M. Długosz, S. Deorowicz, KMC 3: Counting and manipulating k-mer statistics. *Bioinformatics* **33**, 2759–2761 (2017). doi:10.1093/bioinformatics/btx304 Medline
57. H. Li, Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997 [q-bio.GN] (2013).
58. P. Danecek, J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, H. Li, Twelve years of SAMtools and BCFtools. *Gigascience* **10**, giab008 (2021). doi:10.1093/gigascience/giab008 Medline
59. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021). doi:10.1038/s41586-021-03819-2 Medline
60. M. L. Hekkelman, I. de Vries, R. P. Joosten, A. Perrakis, AlphaFill: Enriching AlphaFold models with ligands and cofactors. *Nat. Methods* **20**, 205–213 (2023). doi:10.1038/s41592-022-01685-y Medline
61. J. Brassac, F. R. Blattner, Species-level phylogeny and polyploid relationships in Hordeum (Poaceae) inferred by next-generation sequencing and in silico cloning of multiple nuclear loci. *Syst. Biol.* **64**, 792–808 (2015). doi:10.1093/sysbio/syv035 Medline
62. M. Kimura, *The Neutral Theory of Molecular Evolution* (Cambridge Univ. Press, 1983).
63. J. F. Doebley, B. S. Gaut, B. D. Smith, The molecular genetics of crop domestication. *Cell* **127**, 1309–1321 (2006). doi:10.1016/j.cell.2006.12.006 Medline
64. K. M. Olsen, J. F. Wendel, A bountiful harvest: Genomic insights into crop domestication phenotypes. *Annu. Rev. Plant Biol.* **64**, 47–70 (2013). doi:10.1146/annurev-arplant-050312-120048 Medline

ACKNOWLEDGMENTS

We would like to extend our gratitude to the following institutions and colleagues for providing the essential seed accessions or DNA sources: the Nordic Genetic Resource Center (NordGen), Sweden; the IPK Genebank at Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; the National Small Grains Collection (NSGC) – GRIN at USDA-ARS, USA; Gary J. Muehlbauer, Kevin P. Smith, Brian J. Steffenson and Shane Heinen from the University of Minnesota, USA; Austin Case from Busch Ag in Ft. Collins, USA; Aaron Beattie from the Crop Development Center at the University of Saskatchewan, Canada; David Moody from Intergrain, Australia; and Blakely Paynter from the Department of Primary Industries and Regional Development, Australia. Special thanks to Hannu Ahokas for discussions on the Finnish barley breeding history. The authors acknowledge Research Computing at the James Hutton Institute for providing computational resources and technical support for the 'UK's Crop Diversity Bioinformatics HPC' (BBSRC grants BB/S019669/1 and BB/X019683/1), use of which has contributed to the results reported within this paper. Maëva Bicard is thanked for her help in extracting weather data. **Funding:** Carlsberg Foundation grant CF15-0236 (C.D., B.S.); Carlsberg Foundation grant CF15-0476 (C.D., B.S.); Carlsberg Foundation grant CF15-0672 (C.D., B.S.); Carlsberg Foundation grant CF18-0024 (Y.W., Z.H., E.W.); Carlsberg Foundation grant CF20-0352 (B.S., S.T.); Innovation Foundation grant 20798-00008B (B.S., D.P.); German Federal Ministry of Education and Research (BMBF) grant 031B0190A (N.S); German Federal Ministry of Education and Research (BMBF) grant 031B0884A (N.S); European Union's Horizon 2020 research and innovation program grant 862613 (B.J.); Japan Society for the Promotion of Science (JSPS), KAKENHI, grant JP18H02183 (S.N.); Japan Society for the Promotion of Science (JSPS), KAKENHI, grant JSPS 23H00333 (K.S.); Biotechnology and Biological Sciences Research Council (BBSRC), grant BB/S004610/1 (M.S., R.W.); Biotechnology and Biological Sciences Research Council (BBSRC), grant BB/S019669/1 (R.W.); Scottish Government, Rural and Environment Science and Analytical Services Division (RESAS) grant KJHI-B1-2 (M.B., J.R., R.W). **Author contributions:** C.D., M.E.J., and D. V. designed the study. C.D., M.E.J., Y.W., D.V., G.B.F., Z.G., Q.L., S.N., P.R.P., K.S., B.S., N.S., R.W. and E.W. conceived the study. The project and experiments were coordinated by C.D. and M.E.J. The paper was written by M.E.J., C.D., Y.W., R.W., G.B.F., and E.W. *MKK3* genotyping was conducted by Q.L., E.M., J.R., C.V. and C.D. E.M., M.W.R., H.C.T., and C.V. performed the *MKK3* CN analyses (ddPCR). M.E.J., B.J., S.M.K., F.K., Q.L., Y.W., Y.C., F.C., H.D., Z.H., S.T., D.P., E.W., M.M. and N.S. carried out the *MKK3* pangenome analyses. *MKK3* pan-transcriptome analyses were performed by M.E.J., M.B., Q.L., M.S., and R.W. MKK3 in vitro kinase assays and protein modelling were managed by M.E.J. and J.A.C.S. Breeding and field work for *MKK3* NILs were supervised by D.V., A.B., P.M.L.R., A.L., M.U.S., P.A.P., N.W.T. and C.D. The *MKK3* FIND-IT analysis was conducted by M.W.R., and C.V., M.E.J., K.B.B., E.M. and C.V. oversaw the *MKK3* germination assays, while K.B.B. performed malt analyses. All authors read and provided valuable input on the manuscript. **Competing interests:** M.E.J., C.B.A., K.B.B., J.A.C.S, Z.G., S.M.K., F.K., Q.L., M.U.S., P.R.P., D.P., M.W.R., H.C.T., E. M., N.W.T., S.T., C.V., B.S. and C.D. are current or former employees of the Carlsberg Research Laboratory. All other authors declare no competing interests. **Data and materials availability:** All data are available in the main text or the supplementary materials. Local collection and Genebank accessions are available by SMTA under the International Treaty and the Nagoya protocol. Barley FIND-IT variant seeds can be provided by the Carlsberg Research Laboratory pending scientific review and a completed material transfer agreement as described elsewhere (*33*). Requests for FIND-IT variant seeds should be submitted to Christoph.Dockter@carlsberg.com. **License information:** Copyright © 2025 the authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original US government works. https://www.science.org/about/science-licenses-journal-article-reuse. This research was funded in whole or in part by BBSR and Horizon Europe (grants BB/S004610/1, BB/S019669/1, and 862613), cOAlition S organizations. The authors will make the Author Accepted Manuscript (AAM) version available under a CC BY public copyright license.

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Submitted 6 March 2025; resubmitted 10 July 2025
Accepted 15 September 2025
Published online 6 November 2025
10.1126/science.adx2022

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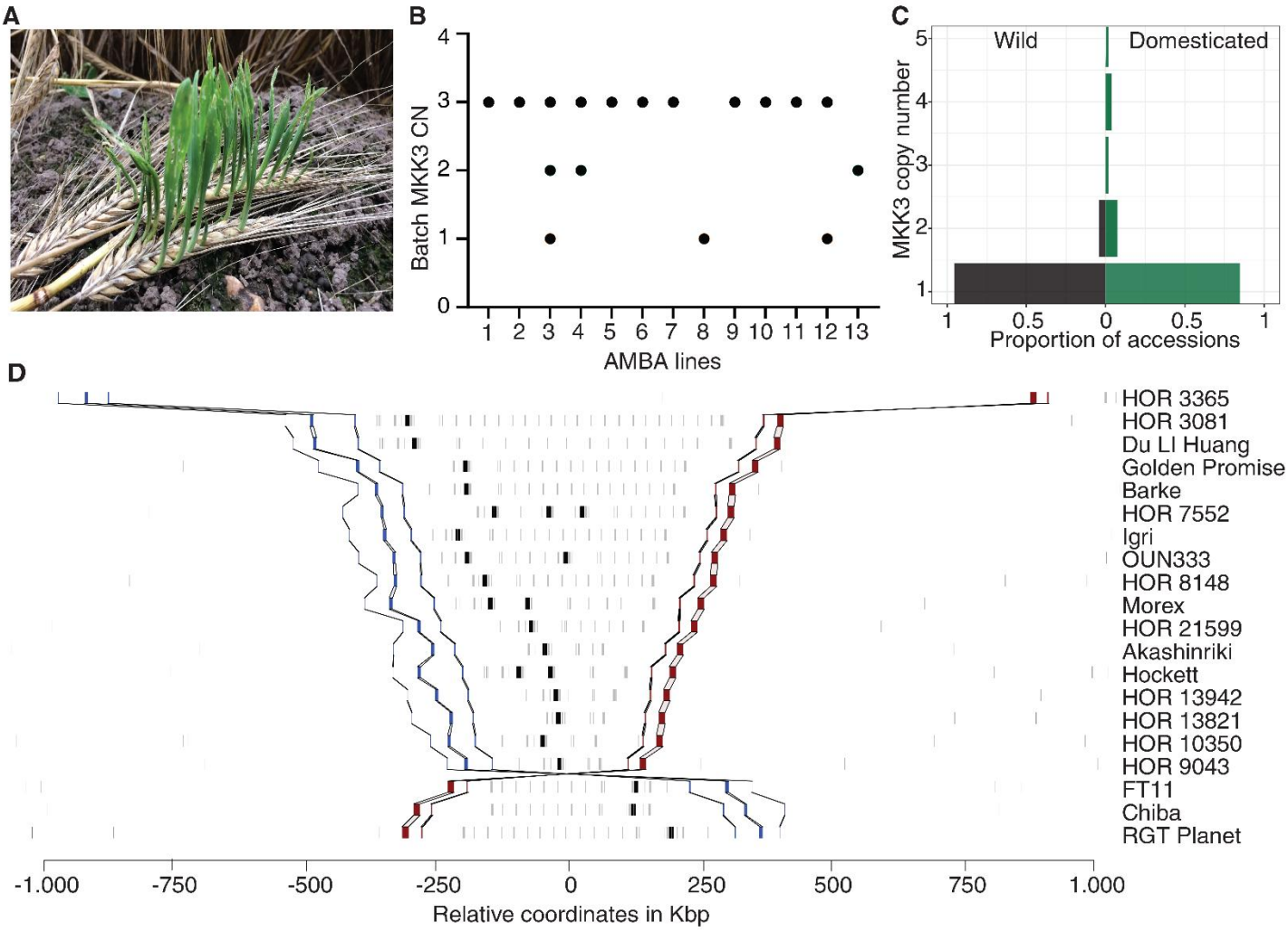

Fig. 1. ***MKK3* locus complexity in barley.** (**A**) Pre-harvest sprouted barley inflorescence. (**B**) ddPCR *MKK3* copy number (CN) analysis across grain batches of AMBA-recommended malting barleys (table S1). (**C**) Distribution of *MKK3* CN (as proportion of wild (23) or domesticated (53) accessions) across 76 pangenome assemblies. (**D**) *MKK3* CNs and structural variations in 20 selected genome assemblies (*28*, *29*). *MKK3* genes in black squares, other genes in grey squares. Red and blue squares denote marker genes that define the synteny, delimit the region and sort the accessions based on the distance between endpoints. Lines connect gene models between different genomes. Accession names are given on the right axis. In three accessions (FT11, Chiba, and RGT Planet), the larger *MKK3* region is inverted (6, 4 and 4 Mb, respectively).

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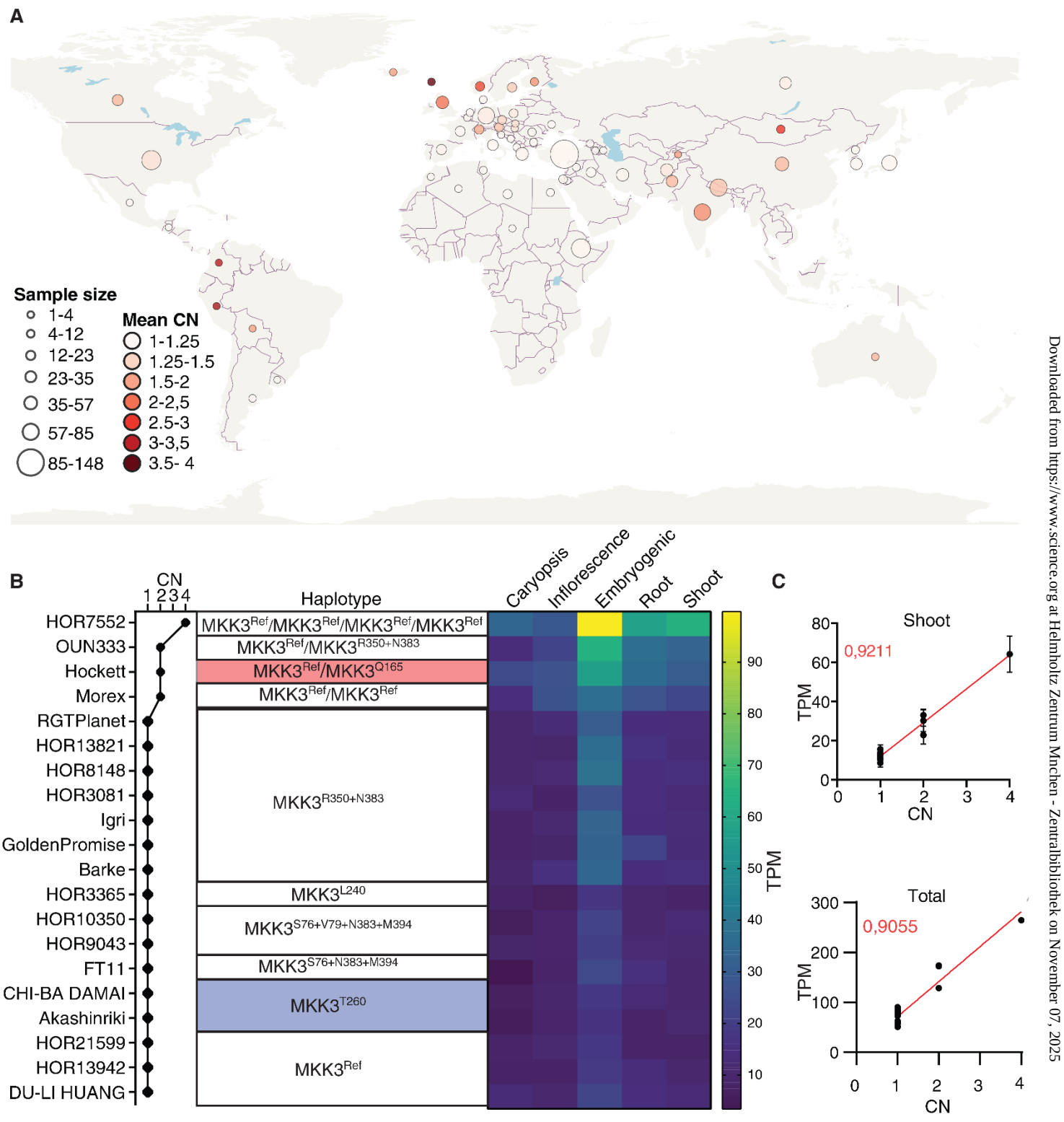

Fig. 2. Global CN distribution of *MKK3* in barley and its functional impact. (A) Mean *MKK3* CN across a global panel of 1,285 geo-localized barley accessions from 68 countries (420 accessions without recorded country of origin from table S3 are excluded). (B) *MKK3* transcript abundance observed by mapping against the PanBaRT20 Reference Transcript Dataset (sum of chr5H56507 and chr5H56511) associated with *MKK3* copy number (CN). (C) *MKK3* transcript abundance in the pan-transcriptome (*29*) shoot tissue as a function of copy number with r^2 value of the linear regression fit in red. Dot size shows sample size (in (A) ranging from 1-148 and in (B) 1-130 barley accessions.

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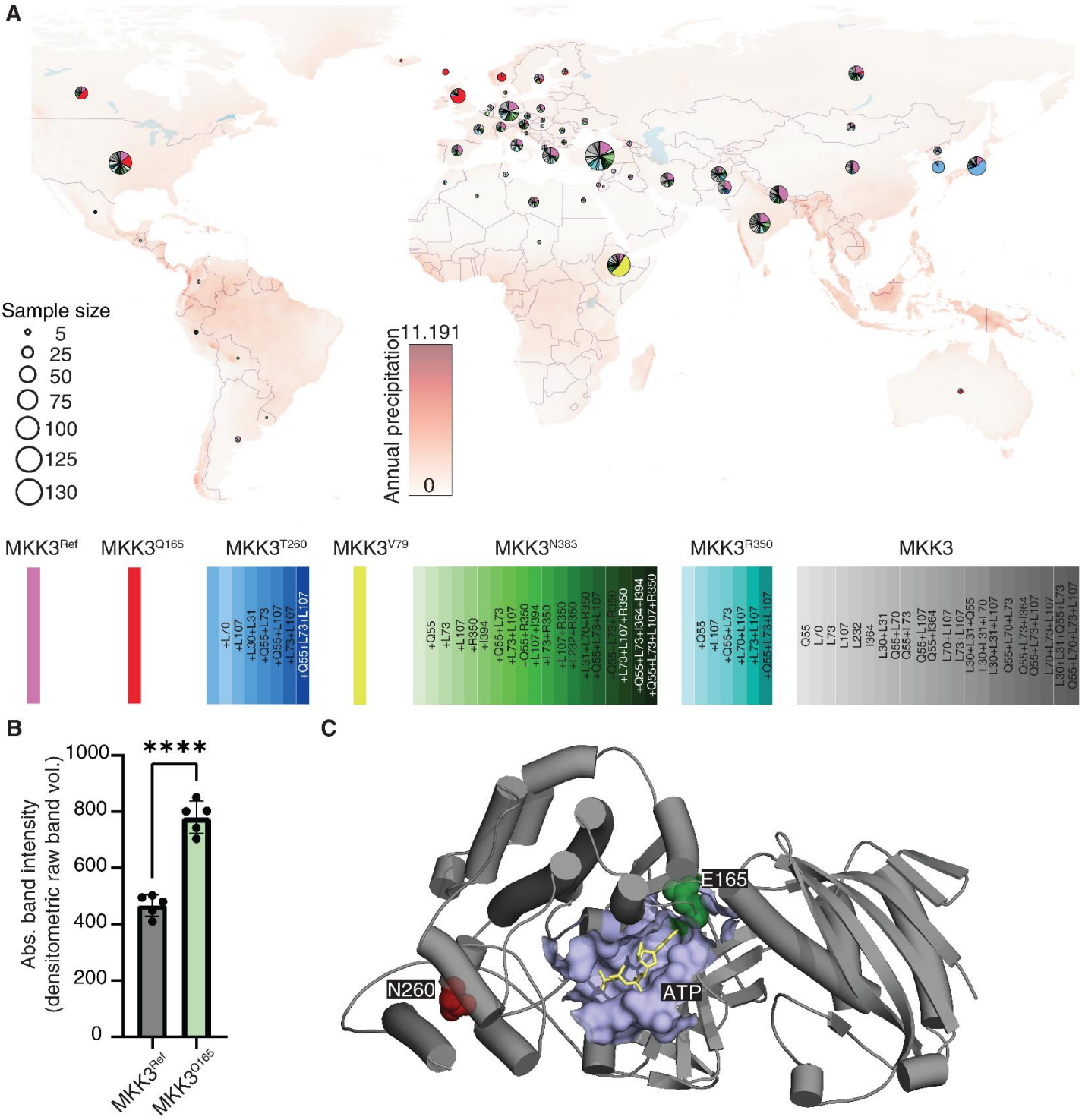

Fig. 3. ***MKK3* haplotype diversity and in vitro kinase activity.** (**A**) Global *MKK3* haplotype diversity across 1,071 geo-localized barley accessions (636 accessions without recorded country of origin, haplotype information or unique *MKK3* haplotypes (1 accession pr. haplotype) from table S3 were excluded). Annual mean precipitation (mm) (WorldClim2.1 BIO12(30)) is shown as a red overlay gradient. Individual MKK3 haplotypes are color coded with $MKK3^{Ref}$ in purple, $MKK3^{Q165}$ in red, $MKK3^{T260}$ containing in blue tones, $MKK3^{V79}$ in yellow, $MKK3^{N383}$ containing in green tones, $MKK3^{R350}$ containing in teal tones and remaining MKK3 haplotypes as a grey tone. Dot size shows sample size. (**B**) In vitro kinase activity of $MKK3^{Ref}$ (table S2) and variant $MKK3^{Q165}$. Error bars are ±SD; Student´s *t* test was used to calculate P values (*P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001; ns, not statistically significant; n=5). (**C**) MKK3 protein model (based on reference $MKK3^{Ref}$) showing amino acid position E165 (green spheres) and N260 (red spheres) associated with changes in MKK3 kinase activity. ATP binding site in kinase domain is shown in light blue with ATP (yellow sticks).

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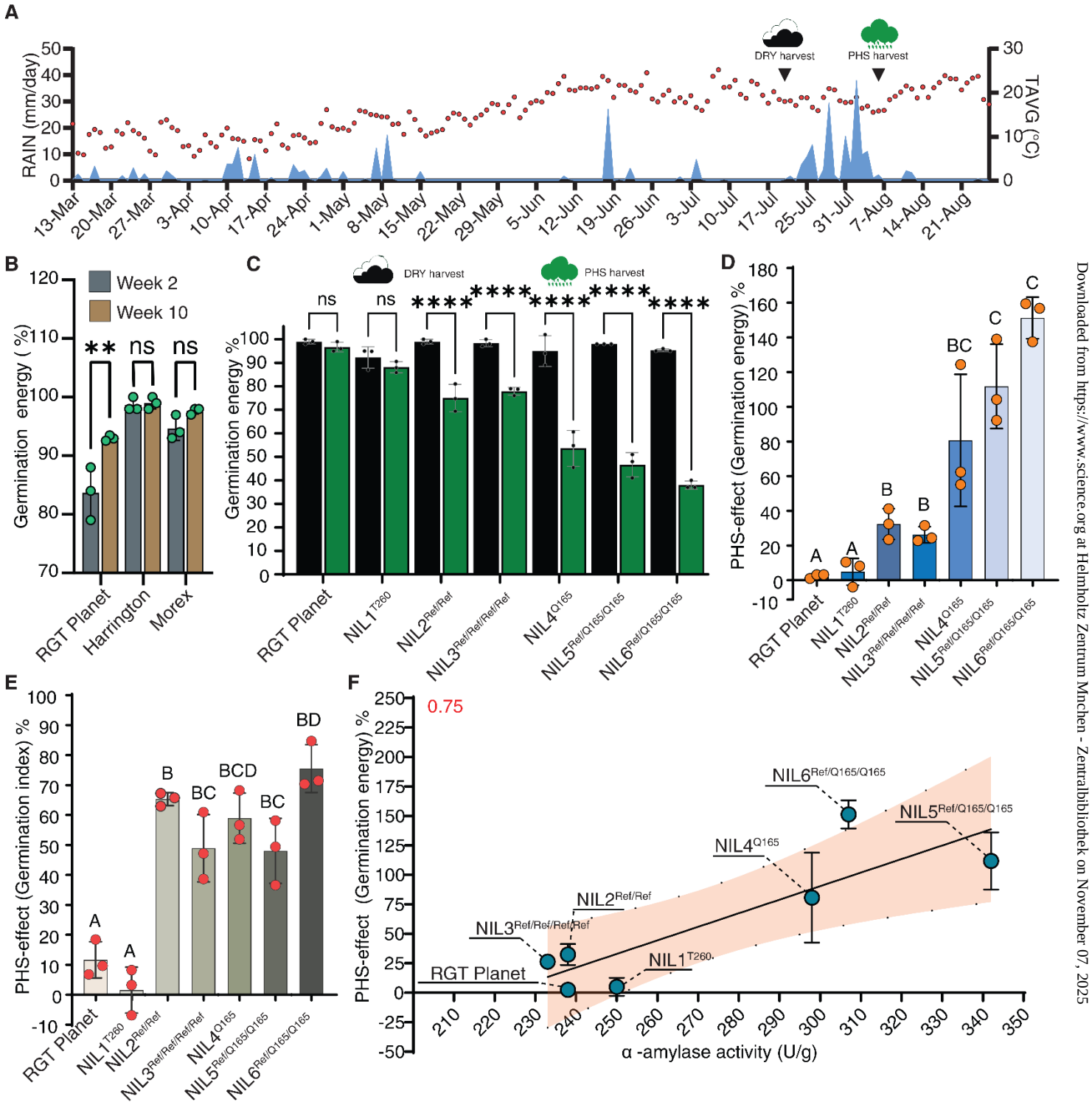

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Fig. 4. PHS field trials and analyses of diverse *MKK3* haplotype NILs. A, Weather data [the average air temperature (TAVC) in degree Celsius in red circles and precipitation (RAIN) in mm/day in blue shading] for PHS field trial site in FRA in the growth season 2023. Marked with arrows are the harvest time points of 'dry' and 'PHS' samples. **B**, Germination energy of *cvs.* RGT Planet, Harrington and Morex at week 2 and 10. Error bars are ±SD; Two-way ANOVA (with Šídák's correction) was used to calculate P values (*P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001; ns, not statistically significant). For each replicate (n=3) 100 seeds from a plot were measured. **C**, PHS trials of *cv.* RGT Planet ($MKK3^{R350+N383}$), $NIL1^{T260}$, $NIL2^{Ref/Ref}$, NIL3 $^{Ref/Ref/Ref/Ref}$, NIL4 ^{Q165}, NIL5 $^{Ref/Q165/Q165}$, NIL6 $^{Ref/Q165/Q165}$ (field-grown, FRA 2023). Shown are grain germination energy (percentage) after *cv.* RGT Planet dormancy is broken. Dry harvest (black bar) and wet harvest (green bar) samples. Error bars are ±SD; Two-way ANOVA (with Šídák's correction) was used to calculate P values (*P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, and **** P ≤ 0.0001; ns, not statistically significant). For each replicate (n=3) 100 seeds from a plot were measured. **D**,**E**, calculated PHS effect (germination energy) (**D**) and PHS effect (germination index) (**E**) of *cvs.* RGT Planet, Harrington and Morex as well as diverse *MKK3* haplotype NILs in RGT Planet background. Error bars are ±SD; One-way ANOVA test (with Brown-Forsythe and Welch correction) **D**) ($p < 0.05$, $F^*(8.000, 4.492) = 33.44$, $p = 0.0012$) and **E**) ($p < 0.001$, $F^*(8.000, 10.22) = 26.16$, $p < 0.001$) was used to compare the effect of diverse *MKK3* haplotypes on PHS effect. Letters denote significant differences between groups. **F**, PHS effect (germination energy, calculated from PHS data shown in (**D**), see Methods) as a function of α-amylase activity of micro-malted field-grown grains (NZL 2024) with r^2 value of the linear regression fit in red.

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