









RESEARCH ARTICLE

Genomic, climatic, and cultural diversity of maize landraces from the Himalayan Kingdom of Bhutan

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Societal Impact Statement

Bhutan is an ancient kingdom in the Himalayan range and one of the most rugged, geodiverse, and mountainous agricultural countries in the world. Historically secluded and geographically isolated, Bhutan is a hotspot for Himalayan agrobiodiversity where small-scale agriculture supports the livelihoods of a large share of the resident population. Here, Bhutanese maize agrobiodiversity is explored to unlock its adaptation potential using genomics and participatory variety selection in combination with climate research. We show that Bhutanese traditional farmers maintain a wealth of diversity that may support the sustainable intensification of maize cropping in the Himalayas and beyond.

Summary

- Bhutan, an ancient kingdom enshrouded in the Himalayas, hosts largely untapped agrobiodiversity that may harness genetic variation useful for adaptation to local climates and user needs.
- Here, we genotyped-by-sequencing 351 pooled samples of local maize (*Zea mays* L.) landraces, the entire collection of the Bhutan National Gene Bank, comparing their genomic diversity with maize from other countries in the Himalayan range. We reconstructed the adaptation of Bhutanese maize to historical and projected climates, identifying areas of future maladaptation. We then run a common garden experiment involving local smallholder farmers in a participatory evaluation of landraces' performance, aiming at the identification of quantitative trait nucleotides (QTNs) contributing to adaptation, performance, and farmers' choice.
- We found that Bhutanese maize agrobiodiversity is unique in the Himalayan range, and a locus on Chromosome 5 supports the differentiation of three distinct genetic clusters. We found that a portion of current genomic diversity can be associated with the Bhutanese landscape and that maize cultivation in the southwest of the country may be negatively impacted by projected climates. We also found that Bhutanese maize agrobiodiversity is large and may contribute to adaptation and

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improvement. A genome-wide association study identified 117 QTNs for climatic adaptation, agronomic performance, and farmers' preferences.

- Our results show that Bhutanese maize landraces are a unique source of genetic agrobiodiversity for local adaptation. We found that the integration of genomics, climate science, and participatory methods can speed up the identification of genetic factors contributing to the sustainable intensification of maize cultivation in the Himalayas and beyond.

KEYWORDS

climate adaptation, genome-wide association study, genomics, participatory variety evaluation, plant genetic resources, *Zea mays*

1 | INTRODUCTION

Maize (*Zea mays* L.) is a very significant crop in global agricultural systems. In industrialized countries, it is largely used as livestock feed and to produce goods including biofuels, sweeteners, and oil (Ranum et al., 2014). It is characterized by high yield potential that is expressed in optimal management conditions, including significant water and fertilizer availability. Due to its high yield, it is particularly attractive to farmers in areas with land scarcity and high population pressure (Shiferaw et al., 2011) but is also a staple in the diets of millions of people in subsistence and smallholder farming systems of Sub-Saharan Africa, Latin America, and South Asia (Grote et al., 2021). Studies have shown that the climate crisis may severely impact maize cultivation, especially in low-resilience farming systems (Omoyo et al., 2015). Increased frequency and intensity of dry spells as well as heavier-than-usual rainfall during crucial stages of growth and development may result in substantially decreased maize yields (Li et al., 2019). While some crop-climate model ensembles estimate a moderate impact of a warming climate on maize yields, the increased frequency of extreme events poses a threat especially in low-resilience, low-input farming systems (Zhang et al., 2022). Recent projections show that maize yields may decrease by up to 24% in major growing regions (Jägermeyr et al., 2021), a bleak perspective when coupled with the two billion rise in the human population by 2050 predicted by the United Nations. To ensure food security, particularly in emerging countries, maize breeding must act now to improve adaptation traits in a changing climate (Fróna et al., 2019).

Plant genetic resources (PGRs) including traditional farmer varieties are crucial in maintaining the adaptability and evolvability of crop gene pools, including resistance to pests, diseases, and abiotic constraints (De Kort et al., 2021). Not only does allelic diversity in PGRs provide the means to support adaptation to current cultivation constraints, but it can also enable breeding to address future needs (Acosta-Quezada et al., 2022; Caproni et al., 2023; Petropoulos et al., 2019; Woldeyohannes et al., 2022). Landraces maintained in farmer fields are selected for adaptation to local environments and local farmer preferences through continued cycles of local planting, harvesting, and selection over time (Bitocchi et al., 2015). Their allelic diversity bears great potential for exploitation in breeding programs

and can now rapidly and effectively be disclosed thanks to modern genomic tools that may unravel molecular mechanisms underlying adaptation and agronomic performance (Corrado & Rao, 2017; Guan et al., 2022).

Maize is truly a global crop, and thousands of maize PGRs are still cultivated throughout the world. They bear adaptability to different conditions and hold great value as a genetic resource for agronomic and quality traits improvement, including resistance to biotic and abiotic stresses (Böhm et al., 2014; Strigens et al., 2013). Archeological and genetic studies show that *Z. mays* originated in Mexico (Yang et al., 2023), and from there spread to the world following different waves of human migration and commerce (Tenailon & Charcosset, 2011; Mir et al., 2013). The current maize global diversity is very high and is contributed by its recent evolutionary history and very dynamic genome (Grzybowski et al., 2023). Still, large maize agrobiodiversity lies untapped in PGRs that are maintained in situ and ex situ. Characterizing this diversity could further enrich the breeding pool for local adaptation traits, shed light on maize history and migration, and improve conservation strategies (McLean-Rodríguez et al., 2021).

Bhutan and Nepal are landlocked countries in the Himalayan range, bordering the northeastern part of India where maize is cultivated all year round (Kandel & Shrestha, 2020; Katwal et al., 2013; Mukerjee et al., 2018). In this region, maize landraces grow from about 0 m above sea level (m.a.s.l.) to almost 3000 m.a.s.l., showing high morphological diversity (Krishna et al., 2020; Prasanna, 2010). Genetic studies using traditional molecular markers revealed the distinctiveness of maize landraces in the Indian Himalayas compared with the rest of the subcontinent (Prasanna, 2010), but the genetic diversity of maize PGRs in this region remains significantly unexplored. Only recently, in 1974, Bhutan opened its borders to international trade and research. A genomic characterization of Bhutanese and Himalayan maize PGRs may allow to access gene pools that have long been hidden and secluded in the pockets of Himalayan mountains, shedding light on maize agrobiodiversity in the region.

In this study, we used DNA sequencing approaches to develop single nucleotide polymorphism (SNP) markers on Bhutanese maize landrace collection maintained at the Bhutan National Gene Bank (BNGB), United States Department of Agriculture (USDA), and The

International Maize and Wheat Improvement Center (CIMMYT) gene banks. We incorporated accessions from the neighboring Nepal and Indian Himalayas to compare their genetic relationship to a larger geographic scale. Further, we assessed the agronomic and climatic diversity and/or adaptation of Bhutanese germplasm in local farming. We found that Bhutanese maize landraces are genetically distinct and have allelic diversity with potential breeding value for climate adaptability and agronomic traits. In addition, we identified accessions that scored high on farmer appreciation. Our findings may contribute to further utilization of these genetic resources in the development of new maize varieties that may play a significant role in ensuring food security in the region.

2 | MATERIALS AND METHODS

2.1 | Plant materials and DNA extraction

We gathered the entire collection of maize landraces from the BNGB, totaling 351 accessions collected in the country between 2005 and 2017. We complemented this collection with five improved lines from BNGB and six landrace accessions of Bhutanese origin conserved at the USDA gene bank (USA). In addition, we obtained 39 accessions from northeastern India representing the Indian Himalayas and 44 accessions from Nepal stored at the CIMMYT gene bank in Texcoco, Mexico. Detailed information on the 445 accessions used in this study is provided in Dataset S1. DNA extraction for the BNGB plant materials was conducted in the BNGB molecular laboratories in Thimphu, Bhutan. DNA of the remaining samples was extracted at the Center of Plant Sciences in Pisa, Italy. The extraction protocol was the same for all samples and aimed at capturing the diversity existing within each landrace. Five seeds per accession were germinated in rolled towels at a relative humidity of 50% and temperature of 22–25°C in the dark. Seedlings were pooled and total genomic DNA was isolated using the GenElute™ Plant Genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's protocol. Therefore, each DNA sample represents a pool of five individual plants. Genomic DNA quality was evaluated in 1% agarose gel and quantified using a spectrophotometer. DNA was sent to IGA Technology Services (Udine, Italy) for library construction and sequencing.

2.2 | Sequencing and SNP calling

Double digest restriction-site associated DNA (ddRAD) libraries were produced using a protocol optimized for maize using *Pst*I and *Bst*YI restriction enzymes to obtain an optimal fragment size distribution (Peterson et al., 2012). Targeted fragments were processed, and the resulting libraries were quantified using Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA) and the Bioanalyzer DNA assay (Agilent Technologies, Santa Clara, CA, USA). The libraries were then processed and sequenced with V4 chemistry paired end 125 bp mode on HiSeq2500 instrument (Illumina, San Diego, CA). Raw reads were

de-multiplexed and checked for quality with FastQC (Andrews, 2010). Because our samples were pooled, we called SNPs with a pooling-aware method implemented in VarScan (Koboldt et al., 2009). Briefly, the demultiplexed reads were filtered using bbdup of BBMap v39.01 (<https://sourceforge.net/projects/bbmap/>) specifying options `qtrim = rl`, `trimq = 25`, and `minlen = 35` to eliminate low-quality and short reads. The reads were mapped against the Maize B73 RefGen_v5 Genome, National Center for Biotechnology Information (NCBI) Bio project PRJEB32225 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_902167145.1/) using BWA-mem v0.7.17 (<https://github.com/lh3/bwa/releases/tag/v0.7.17>) with default parameters. The Samtools v0.1.19 (<https://github.com/samtools/samtools>) was used for conversion to bam files with a minimum mapping quality of 30 and the creation of a multi-bam pileup file. The latter was then used to call variants with the `mpileup2snp` function of VarScan v2.4.6 (<https://github.com/dkoboldt/varsan/releases>). VarScan variants were filtered to retain loci with at least 75% genotype information among all samples and a maximum observed heterozygosity of 80%. We retained only high-quality (QUAL > 30.0) biallelic SNPs. To further increase the quality of markers to be retained in the downstream analyses, we focused on the allelic depth (AD) for each marker. To this end, we set all allele calls supported by an AD lower than 10 (i.e., one copy per each chromosome in the pool) as missing data. We then performed a new round of filtering of markers with missing data >20% and retained only samples with >80% data. Within-pool heterozygous loci were used to compute a minor allele frequency (MAF) for each accession. Finally, we selected markers with an MAF (MAF > 0.01) to undergo diversity analyses. The diversity analyses and genome-wide association study (GWAS) was run on SNP markers with MAF > 0.01 while gradient forest (GF) analysis to estimate the vulnerability of Bhutan maize to future climate was analyzed using MAF > 0.1 following Rhoné et al. (2020). Sequencing reads are available at NCBI (<https://www.ncbi.nlm.nih.gov>) under BioProject PRJNA825653.

2.3 | Genetic diversity analyses

All analyses were conducted in R (R Core Team, 2021). We used a discriminant analysis of the principal components (DAPCs) implemented in R/adegenet (Jombart et al., 2010) to identify the presence of genetic groups among Himalayan samples. The same package was used to produce a neighbor-joining phylogeny and a principal component analysis (PCA) on the SNPs dataset (MAF > 0.01). To assess the genetic distance existing between the genetic clusters, pairwise F_{st} values were estimated using R/hierfstat with the Weir and Cockerham estimator (Weir & Cockerham, 1984). Thereafter, analyses focused on accessions from Bhutan. R/OutFLANK (Whitlock & Lotterhos, 2015) was used to identify candidate loci under selection (i.e., outlier loci) among the different genetic groups in the country, testing each against all the others, according to a false discovery rate (q value) of 5% (Storey, 2003). Gene models and associated protein-coding genes and gene functions were also searched within ±1 Mb of the loci.

2.4 | Spatial and bioclimatic characterization of Bhutanese materials

GPS sampling locations of Bhutanese landraces were obtained from the BNGB passport data. Sampling points were projected onto the map of Bhutan using R/raster (Hijmans, 2015) and altitudes of sampling points were derived using the Consortium of International Agricultural Research Centers- Shuttle Radar Topography Mission (CGIAR SRTM) database at 90 m resolution (Jarvis et al., 2008). All climate analyses were restricted to the country shape of Bhutan. Historical climate data was derived from fifth generation atmospheric reanalysis version data (ERA5) released under the Copernicus Climate Change Service (Hersbach et al., 2020), covering 30 years (1981–2010) at a spatial resolution of 0.25°. Future climate projections were derived from daily climate data extracted from a selection of models among the 38 available at two Representative Concentration Pathway (RCP), namely, RCP 4.5 and RCP 8.5, at the horizons of 2050 and 2070. Multiple General Circulation Models (GCM) share model parametrization, forcing and validation data, which can lead to the potential issues of biases in the mean and variance, overestimation of sample size, and the possibility of false correlations (Altinsoy & Kurnaz, 2021; Ruane & McDermaid, 2017; Sanderson et al., 2015). To reduce these problems, we chose seven models that demonstrated relative independence and had better skills compared with historical observation data. By doing so, we were able to select a subset of models that did not replicate each other, ultimately reducing codependency and bias. The selected models were used to prepare multi-model ensembles (MMEs) for future projected climates with the same spatial resolution as historical data.

Historical and predicted future climate data were used to derive 19 biologically meaningful indicators and bioclimatic variables using R/dismo (Hijmans et al., 2015). These 19 indicators describe seasonal trends as well as limiting climatic factors for plants (Dataset S2). Collinearity among the derived bioclimatic variables was measured with functions implemented in R/BiodiversityR (Kindt & Coe, 2005). For the following analyses, non-redundant bioclimatic variables with a variance inflation factor (VIF) < 10 were the only used.

2.5 | Estimation of genomic offset to future climate scenarios

A GF machine-learning tree-based approach was used to model the distribution of Bhutanese maize landraces to historical climate using R/gradientForest (Ellis & Smith, 2012). The GF model was trained using non-collinear historical bioclimatic variables and eigenvectors of a spatial weighting matrix (namely, Moran's eigenvector maps [MEMs]) at sampling points as predictors and SNPs with MAF > 0.1 as response variables. The model was built as a forest of 500 trees at each SNP and was used to assess the mismatch between the genetic composition we observed using historical bioclimatic variables as predictors and the expected genetic composition at future climate scenarios and horizons with a method derived from Caproni et al. (2023). The genomic offset was estimated as the Euclidean distance between

the allelic turnover predicted using historical and projected climate datasets (Fitzpatrick & Keller, 2015).

2.6 | Common garden experiment

A subset of 142 Bhutanese landraces collected from the BNGB was grown in a common garden experiment to evaluate phenotypic performance in a participatory research framework. The subset was selected based on geographic representation from 17 out of 20 *Dzongkhags* (districts), 98 out of 205 *Gewogs* (sub-districts) and the inclusion of diverse vernacular names based on regional folk classification and variety naming system (Dataset S1). Accessions were grown during the cropping season of 2018 (from April to August) at the land of Farm Machinery Corporation Limited (FMCL) based in Norzinthang in Trashigang Dzongkhag, eastern Bhutan, which stands at an elevation of 1004 m.a.s.l. The experiment was set up in an area of 0.05 acre. The dimension of each plot which consisted of one accession was 4.8 m² (4 m × 1.2 m) with a spacing of 60 × 20 cm (60 cm between row to row and 20 cm between plant to plant). Each plot was comprised of two rows, each consisting of 20 plants, to a total of 40 plants per plot. Two locally improved varieties; *Bhur Ashom 1* and *Yangtsipa* were planted in six standard check plots across the field layout for each replicate. In addition, they were planted at the experimental field periphery acting as a buffer. Phenotypic traits were recorded on five out of the 40 plants per plot, selected randomly with no more than three plants in each row. To control variability and increase the reliability of our findings, the experiment was run in two replications following a randomized complete block design.

A total of seven quantitative traits were recorded: plant height (PH) in cm, ear height (EH) in cm, ear length (EL) in cm, the number of kernel rows/ear (KRW), length of growing cycle (ELY), grain yield per plant (GYP), and 100 kernel weight (HKW). Plant height was measured from the ground level to the peduncle just above the flag leaf, regardless of tassel morphology. This was done after the milk stage when the height maximum is near and/or complete. EH was measured from the base of plants to the node of the topmost ear. To assess the length of the growing cycle/earliness, we devised an earliness scale (ELY) based on the percentage of tasseling and silking observed between Days 80 and 90. Accessions were scored on a scale of 1 to 6 whereby, 1 = *very early* (100%), 2 = *early* (85%), 3 = *intermediate* (50%), 4 = *late* (30%), 5 = *very late* (10%), and 6 = *extremely late* (0%). Grain yield was calculated based on published guidelines (Bajo, 2004).

2.7 | Focus group discussions and participatory evaluation of genetic materials

Twenty maize-growing farmers participated in the focus group discussions (FGDs) and evaluation of the accessions. Farmers came from five local *Gewogs*: Kanglung, Samkhar, Yangneer, Khamdang and Bartsham. Khamdang is located in Trashiyangtse *Dzongkhag* while the other four *Gewogs* are found in Trashigang *Dzongkhag*. These *Gewogs*

were selected for their nearness to the study site, accessibility, and availability of farmers, and represent contrasting maize growing ecologies of the eastern part of Bhutan where maize is mainly grown. Trashiyangtse is mainly cool temperate to warm temperate while Trashigang is warm temperate to dry subtropics. Participants were selected maintaining gender balance and a number of participants from each Gewog. Farmers from each Gewog were accompanied by their respective Agriculture Extension staff who had experience in guiding previous participatory research. FGDs were conducted before field evaluations. For this task, farmers were divided into two main groups based on gender to capture as much information as possible. Participating farmers were asked to list down the maize traits that are of interest to them based on their farming experience and knowledge. The farmers were then tasked with assessing the 142 maize accessions in the field. The participatory evaluation coincided with the seed maturation of most accessions at a time when differences in phenology were still visible. The 20 farmers and 6 Agriculture Extension Officials were organized into 5 groups comprising two groups of men with 4–5 farmers per group and three groups of women with 3–5 members per group. One Agriculture Extension Officer was allocated to each group to guide the farmers in this exercise. Groups then moved into the field from random entry points and were asked to evaluate each accession for their overall appreciation (OA) on a Likert scale from 1 (*very low*) to 5 (*very high*). The scoring of each farmer for each of the 142 accessions was independently recorded.

2.8 | Phenotypic data analysis and association study

Phenotyping data was analyzed using R using custom scripts. R/corplot (Taiyun Wei et al., 2021) was used to study the correlation among phenotypic traits. Graphs for quantitative data were generated using R/ggplot2 (Wickham, 2016). A PCA was performed to estimate the relative importance of different traits in capturing variation in the collection and to establish the relationship among all variables under study using R/Adegenet (Jombart et al., 2010). Analysis of variance (ANOVA) was executed in R/Agricolae (Mendiburu, 2019) to examine the impact of the randomized complete block design on trait variation. Further, a linear mixed model was adopted and analyzed in R using the package lme4 (Bates et al., 2015). The linear mixed model was $\text{model} = y \sim (1|\text{genotype}) + (1|\text{replicate}) + (1|\text{replicate: plot})$, where y represents the vectorized trait data collected. Best linear unbiased predictor (BLUP) values were generated and utilized in downstream analysis. Heritability estimates and covariance estimates were obtained from the mixed linear model. Narrow-sense heritability (h^2) was calculated as the ratio of the additive genetic variance to the total phenotypic variance.

A GWAS was performed on phenotypic traits using BLUP values, participatory evaluation traits, and bioclimatic variables. The GWAS was run using a fixed and random Circulating Probability Unification (FarmCPU) model (Liu et al., 2016) implemented in the package in R/GAPIT (Lipka et al., 2012) using the first PC calculated on genotypic

data as a covariate. Marker-trait associations/quantitative trait nucleotides (QTNs) were defined when SNPs surpassed the significance threshold of a false discovery rate of 0.05 based on Storey's method (Storey & Tibshirani, 2003). GWAS results were plotted with the R package qqman (Turner, 2018). The reported Manhattan plots display a stringent Bonferroni threshold corresponding to a nominal p -value of .05 to aid the identification of the most significant SNPs. GWAS associations were compared with the output of outlier loci detection, using LD measures as a reference framework. We searched for potential candidate genes in the vicinity of genomic associations using the Zm00001d.2 Filtered Gene Set from Maize B73 RefGen_v5 Genome, MaizeGDB (<https://maizegdb.org/genome/assembly/Zm-B73-REFERENCE-NAM-5.0>).

3 | RESULTS

3.1 | Maize diversity in the Himalayan regions reflects geographic diversity

The genotyping-by-sequencing of 445 Himalayan maize accessions, including 357 landraces from Bhutan, produced more than 1.36 Bn Illumina paired-ended reads. Reads were aligned to the maize reference genome resulting in 47,364 high-quality SNP markers distributed on the entire genome. Maize landraces in the Himalayas are cultivated as open-pollinated varieties, meaning that some degree of genotypic variation among individual plants belonging to the same accession/landrace is expected. In this study, we pooled five plants for each accession to capture the maximal genetic makeup of our core collection. This required further filtering of the SNP set to be used to describe the diversity in the collection (Dataset S3). The sample with the highest number of heterozygous SNPs was at 50% and the lowest at 2% of the total (Dataset S3). On average, the within-pool minor allele frequency (MAF) was 0.22, approximately one in five. There is a general trend whereby samples with a higher number of heterozygous loci also had higher MAF (Figure S1). The filtered set of SNPs to be used in analyses downstream was comprised of 27,198 genome-wide markers.

Maize landraces included in this study were collected from the entire geographic span of the Himalayas, from 21.1 to 34° latitude and from 72.7 to 92.1° longitude (Figure 1a,b). To summarize maize diversity across the landscape, we conducted a DAPC that unveiled four genetic clusters best describing the allelic frequencies across the Himalayas (Figure S2). Clusters 1, 2, and 3 grouped accessions from Bhutan, while Cluster 4 grouped accessions originating from Nepal and India (Figure 1a). Within Bhutan, the three genetic clusters were influenced by geography, Cluster 2 being mostly found in the southern foothills to the southwest, while Clusters 3 and 1 being more characteristic of the East and the highlands of the country (Figure 1b). A PCA computed on SNP data shows that Bhutan accessions are separated from India and Nepal (Figure S3). The first four principal components were considered based on a screen plot (Figure S4), PC1 and PC2 show the clearest separation between the clusters (Figure S5).

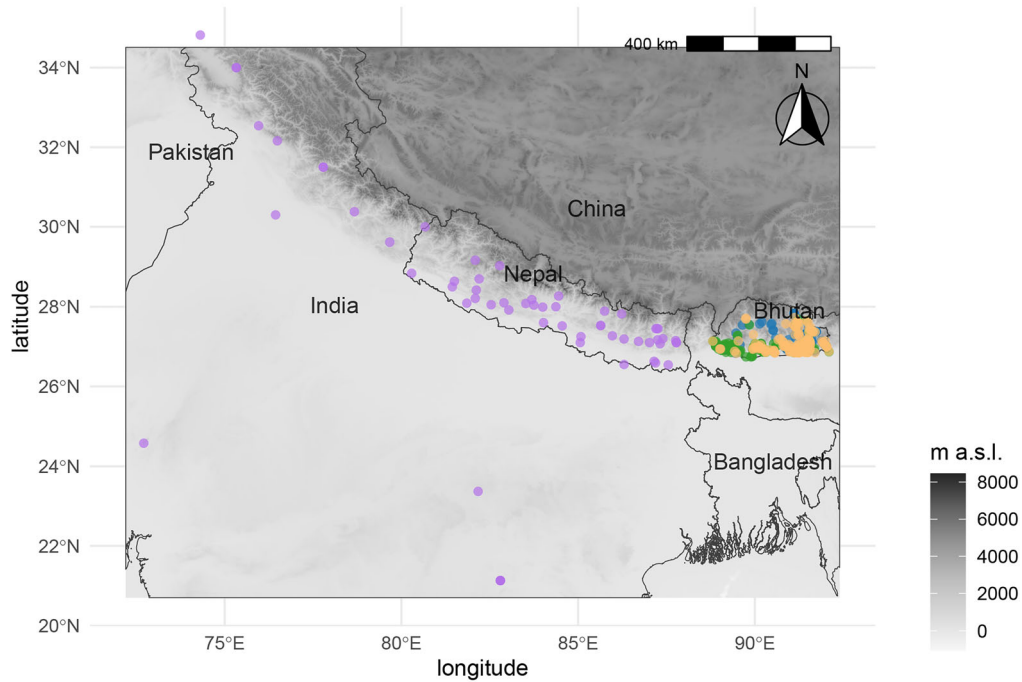
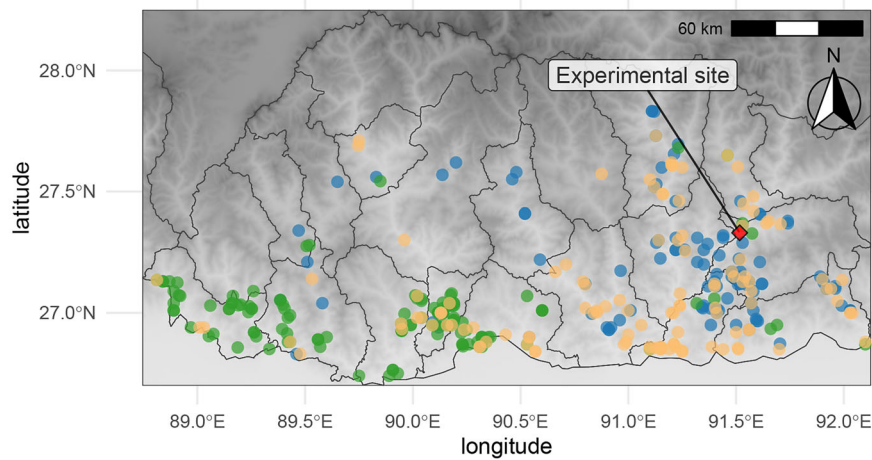
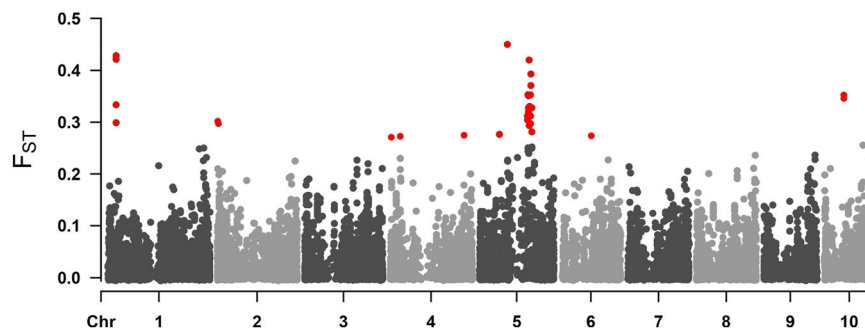
(a) Eastern Himalayas**(b)** Bhutan**(c)** DAPC cluster 2

FIGURE 1 Legend on next page.

PC1 and PC2 explained 6.76% and 3.76% of the variance, respectively, reporting overall low structure (Figure S3).

We then focused on the group of Bhutanese accessions. Pairwise F_{st} distances were rather low between Bhutanese clusters, the most reciprocally isolated being Clusters 1 and 3 ($F_{st} = 0.073$) (Dataset S4). Most accessions in Clusters 1 and 3 were sampled from eastern Bhutan, spatially closer than Cluster 2 with any of them (Figure 1b). We explored the presence of outlier loci, putatively under selection, comparing each genetic with the rest samples. Thirty-one outliers were detected from Cluster 2, 18 of which were observed on Chromosome 5 (Figure 1c; Dataset S5), some of which mapped in regions harboring several annotated genes (Dataset S6). There were no outlier loci observed in Clusters 1 and 3.

3.2 | Bioclimatic characterization of Bhutanese maize landraces in a changing climate

A correlation analysis between bioclimatic variables and altitude in Bhutanese maize sampling points showed a moderate positive correlation with variables that measure the range between maximum and minimum temperatures including bio2 (diurnal range), bio4 (seasonal temperature change in a year), and bio7 (range in the warmest and coldest month) (Figure S6; Dataset S2). Meaning that the temperature range expands with rising altitude. Nevertheless, we note that accessions in this study grow in highly heterogeneous altitudes. These variables also show a negative correlation with all precipitation variables bio12 to bio19. All precipitation variables were negatively correlated with altitude, following the south–north altitudinal gradient in the country going from the southern foothills to the greater Himalayas. Temperature-related variables bio6 (lowest temperature of the coldest month), bio9 (average temperature of the driest quarter), and bio11 (average temperature of the coldest quarter) showed a strong positive correlation with annual precipitation variable bio12 (average total rainfall in a year).

To assess the adaptability of Bhutan maize landraces to future climate-projected scenarios, we developed a GF model using historical climate and MEMs as predictors and 13,469 SNPs ($MAF > 0.1$) as response variables. We found that the change in the allelic turnover in the maize collection was best predicted by MEM1, describing the spatial distribution, followed by precipitation seasonality (bio15) (Figure 2a). The GF model was then used to estimate climate-driven genomic variation across the landscape (Figure 2b,c). Among the SNPs tested as response variables, about 50% ($n = 6815$) were predicted by

the model (with $R^2 > 0$). Based on the current landscape-genome relations modeled through GF, we estimated the expected genomic composition at different RCP scenarios and horizons. We then derived the future maize genomic offset as the difference between the current genetic variation and the genetic variation that would be needed under a changed climate for each of the tested scenarios. At all RCP-horizon combinations, we observed the highest offset in the southwestern Bhutan, where genetic Cluster 2 is mostly found (Figure 2d; Figure S7).

3.3 | Phenotypic and farmer participatory characterization of Bhutanese landraces

Phenotypic traits were measured in a replicate common garden experiment on a subset of the BNGB materials. The ANOVA statistical model incorporated genotype, replication, and spatial blocking factors to assess their influence on the phenotypic trait variation. Significant differences were observed for all factors across some traits including plant height (PH), ear height (EH), earliness (ELY), grain yield per plant (GYP), and ear length (EL) (Dataset S7). This analysis prompted the adoption of a mixed linear model to further explore and account for the sources of variability in the studied traits. Among the covariance components, the genotype covariance exhibited the highest value for all traits (Dataset S8). The estimated narrow-sense heritability (h^2) ranged from 0.4 (hundred kernel weight [HKW]) to 0.7 (earliness [ELY]). The coefficient of genetic variation (CV_G) for EH and PH was 22% and 12%, respectively (Dataset S8). The BLUPs were employed in subsequent analysis.

Although the traits showed a wide variation, the distribution of all traits was normal (Figure S8; Dataset S8). A correlation between traits exhibited a high positive correlation of EH with PH (Figure S9). ELY was positively correlated with PH, EH, and EL. KRW and HKW had a negative correlation. A PCA on phenotypic traits did not reveal the existence of any structure within the core collection based on either of the DAPC clusters observed with genotypic data (Figure S10).

Farmers participating in FGDs highlighted preferred traits for maize cultivation (Dataset S9). Participatory field evaluations further captured gender differences in these preferences. The OA of genotypes score between men and women was significantly different ($p < .0001$) as revealed by a paired t -test (Figure S11). This is ascribed to the fact that men and women, in some instances, value traits differently when performing their selection (Dataset S9). Only women

FIGURE 1 Geographic distribution and genetic clustering of Himalayan maize. (a) Collection points across Bhutan, India, and Nepal. (b) Enlarged map showing accessions from Bhutan. The longitude and latitude values in WGS84 EPSG:4326 degrees are reported on the x-axis and y-axis, respectively. Samples without global positioning system (GPS) coordinates are not shown. The sampling area is reported in shades representing altitude according to the bar on the side (meters above sea level, the color scale is the same in panels a and b). Colors represent each sample's genetic discriminant analysis of principal components (DAPCs) clusters (1–4). The location of the common garden experiment is shown with a red diamond. (c) Plots show outlier loci (highlighted in red) based on fixation index (FST) distribution (y-axis) across all chromosomes (x-axis). Outlier loci were selected based on values of q -value of 0.05 and $He > 0.1$. DAPC Clusters 1 and 3 are not shown.

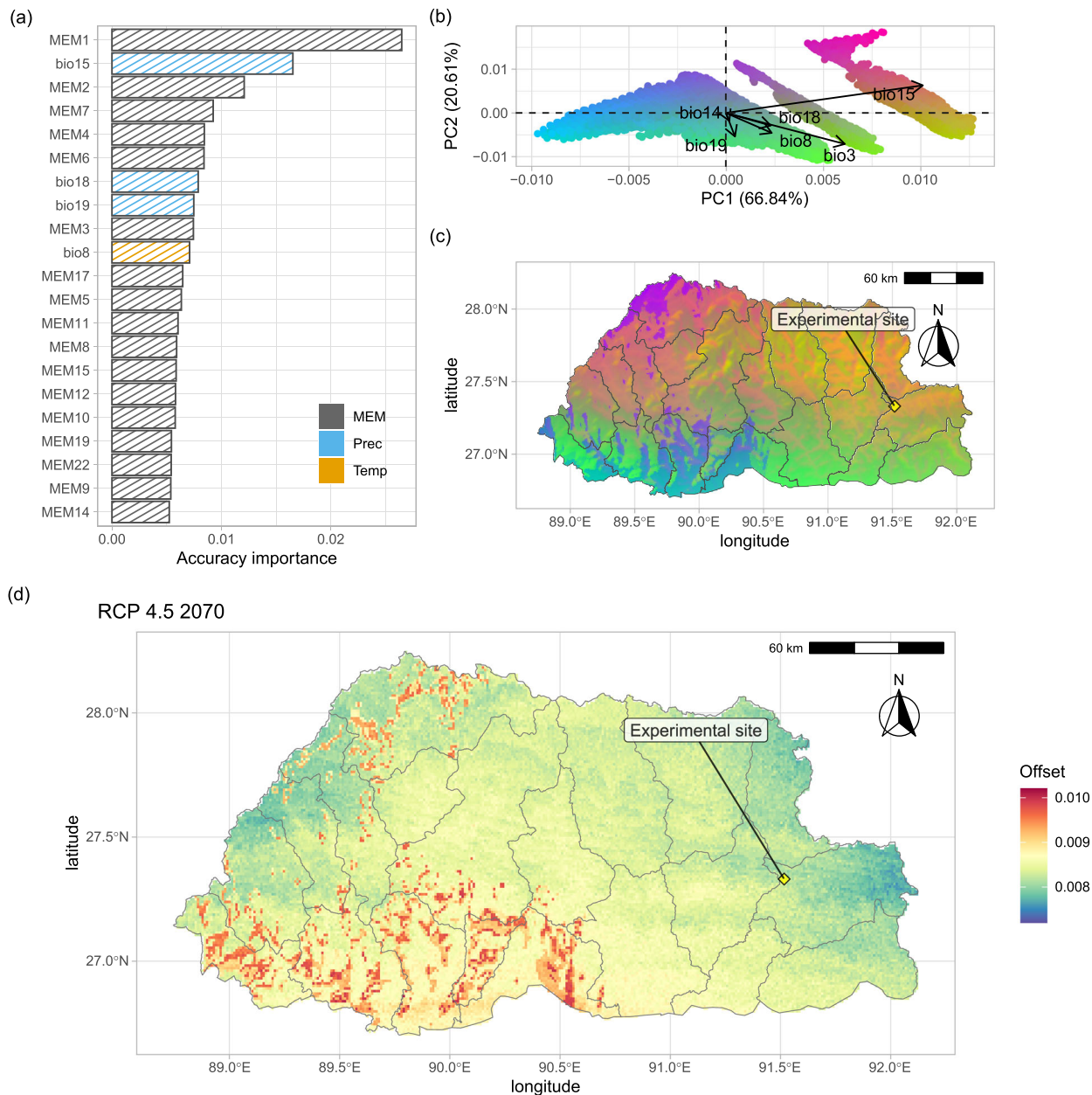


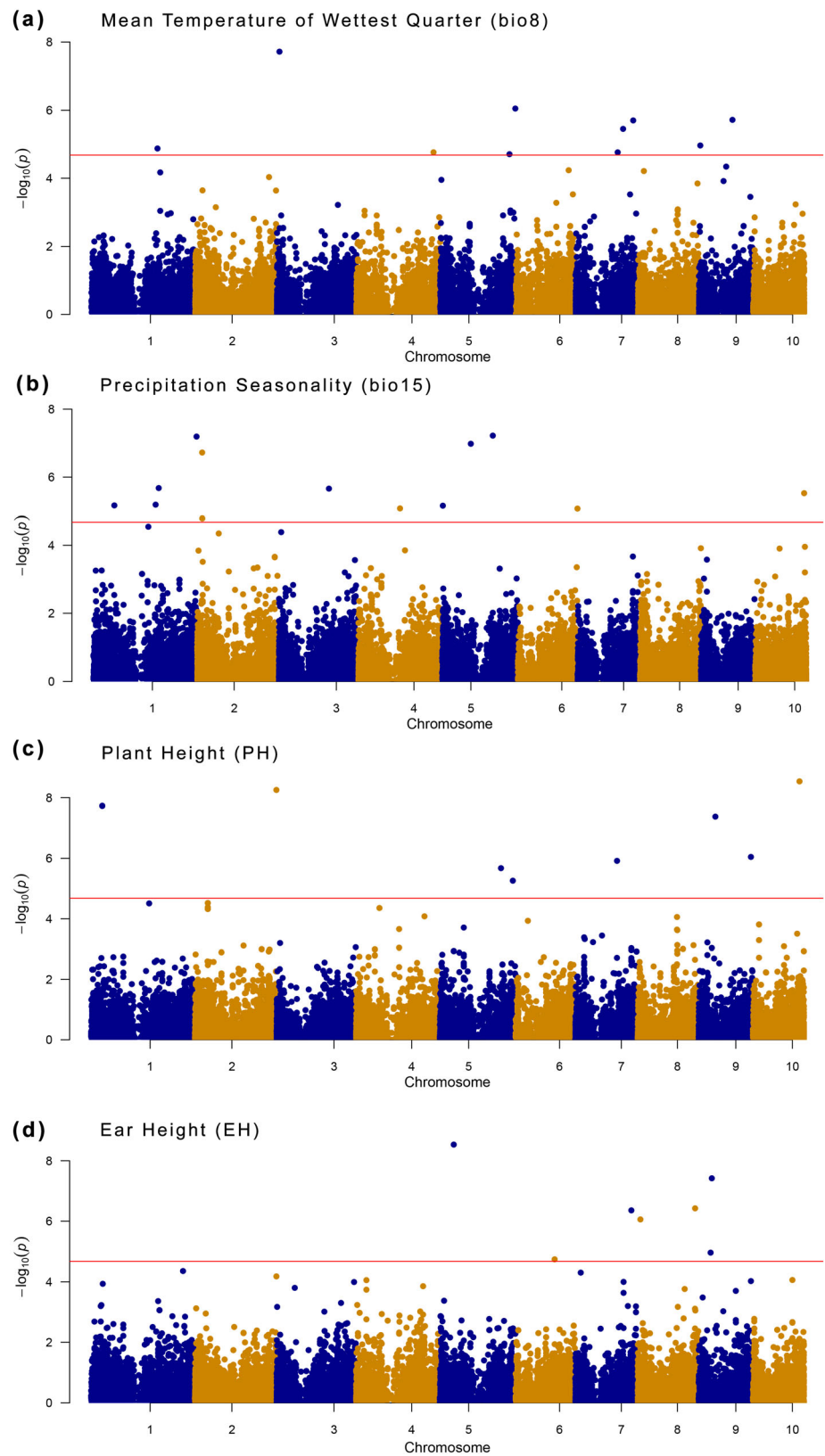
FIGURE 2 Bioclimatic and spatial diversity explain genomic variation in the distribution of Bhutanese maize landraces. (a) Ranked accuracy importance of the bioclimatic and spatial variables based on the gradient forest (GF) model. Bioclimatic variables (bio) include temperature (temp) and precipitation (prec). Spatial variables are based on Moran's eigenvector maps (MEMs). (b) Biplot of the biological space, represented by principal components of the transformed grid with an RGB (red, green, blue) color palette relative to its first three dimensions. (c) GF-transformed bioclimatic variables across Bhutan. Colors based on bioclimatic-genetic space in (b). (d) Genomic offset based on Representative Concentration Pathways (RCPs) 8.5 ensemble climate projections at the horizon of 2070. The color scale indicates the magnitude of the mismatch between current and projected climate-driven turnover in allele frequencies. The location of the common garden experiment is shown with a yellow diamond in (c) and (d).

highlighted their preferences for varieties with ease of shelling, suggesting their role in post-harvest activities. Men preferred varieties with delayed senescence of stalks to provide fodder for the animals throughout the cropping season. From the discussion, we garnered that farmers' preferences/selection are driven by observation of quantitative traits including plant height, kernel color, ear length/ear size, and earliness. This was confirmed by the quantitative analysis of OA

scores which showed a positive correlation with PH, EL, KRW, HKW, and GYP (Figure S9).

The top 50 accessions according to farmers' evaluation were selected from the genotypes subjected to participatory evaluation (Dataset S10). Farmers' evaluations were combined with phenotypic data to prioritize genetic materials for the potential for local adaptation. From the phenotypic data collected, we selected accessions with

FIGURE 3 Genome-wide association study using single nucleotide polymorphism (SNP) markers derived from Bhutan maize landraces. The panels show Manhattan plots for (a) bioclimatic variable bio8 (mean temperature of the wettest quarter) (b) bioclimatic variable bio15 (precipitation seasonality) (c) phenotypic traits plant height (PH) and (d) ear height (EH). The Manhattan plots report individual SNPs across all chromosomes (x-axis) and $-\log_{10} p$ -value of each SNP association (y-axis). The horizontal lines represent a stringent Bonferroni threshold for a nominal p -value of .05.



desirable trait values guided by the FGD findings: an intermediate plant height of 125–190 cm, an early to intermediate maturity time, an ear length (EL) of 10 cm and above, a grain yield of above 6 g per ear, and 100 kernel weight (HKW) of more than 10 g. Yellow or

orange kernel colors were also selected. Based on these criteria, a total of 30 accessions could be identified (Dataset S11). We then superimposed 50 accessions that ranked highly from farmer participatory evaluation with 30 accessions that ranked highly based on

agronomic trait data. Fourteen accessions overlapped between these two methods of evaluation (highlighted in Dataset S1). Although farmers selected accessions based on phenotypic traits, their OA is also guided by traditional knowledge not captured in the phenotypic data and reinforces the need to integrate these two approaches of variety selection.

3.4 | Genomic associations with climate, traits, and farmers' preference

A GWAS was performed focusing on phenotypic data and bioclimatic data relative to the sampling origin of accessions (Figure 3; Figure S12; Dataset S12). The GWAS yielded strong association signals as evidenced by model fits reported by QQ plots (Figure S13).

GWAS based on bioclimatic data revealed a total of 110 putative QTNs, 24 of which were common among bioclimatic variables (Dataset S13). This redundancy is expected as some bioclimatic variables are derived from the same underlying parameters (Dataset S2), for instance, a QTN at 223.1 Mb on Chromosome (chr) 5 was common across five temperature-related bioclimatic variables including bio1, 5, 8, 10, and 11. We assessed genomic associations with altitude and 11 QTNs were associated with altitude 29.7 Mb on chr1, 9.5 Mb on chr2, 93.4 Mb on chr2, 240.4 Mb on chr4, 223.1 Mb on chr5, 174.5 Mb on chr6, 171.3 Mb on chr7, 179 Mb on chr8, 75.2 Mb on chr9, 117 Mb on chr10, and 144.9 Mb on chr10. There were 5 QTNs associated with bio8 and 13 QTNs with bio15 which were among the most important climatic drivers of the GF model (Figure 3a,b; Dataset S13).

Further, we identified QTNs associated with bioclimatic traits and also determined outlier loci for DAPC Cluster 2 (Dataset S13). Two loci on Chromosome 5 at 148.2 Mb were associated with variables bio12 and bio19 and 150.5 Mb associated with bio15 were identified.

For phenotypic trait data, 11 unique QTNs surpassed a multiple testing corrected significance threshold (Dataset S14). There were six QTNs associated with PH, four for EH, and one for ELY.

4 | DISCUSSION

Maize is one of the most important staples for hundreds of millions of smallholder farmers in Asia (Mir et al., 2013). Although a pre-Columbian arrival of maize in Asia is a suggestive hypothesis, there is no definitive genetic or archeological evidence supporting it. Maize cobs are depicted in sculptures in Indian temples dated around the 13th century (Johannessen & Parker, 1989), and maize illustrations appeared in Chinese literature only since the 1500s (Uchibayashi, 2005), which is compatible with a post-Columbian introduction. It is widely accepted that the introduction of maize into India precedes that of China, and from there it spread throughout Asia. Understanding maize post-domestication movement may contribute to the identification of alleles not considered in breeding programs or conservation (Mir et al., 2013). In these regards, the Himalayan region is very interesting for historical and bioclimatic reasons. We report

that Bhutanese maize landraces are genetically distinct from accessions in the neighboring Nepal and India (Figure 1a,b), suggesting the presence of unique alleles possibly driven by ecological adaptation (Schluter & Conte, 2009) and/or unique traditional practices. The introduction of maize alongside other commodities into the Bhutan region may have been facilitated by Buddhist missionaries who traveled across the Himalayas from the 8th century onwards (Meinert & Sørensen, 2020; Thapa, 1966).

When approaching the study of genotypic diversity in traditional landraces, which are cultivated as open-pollinated varieties, we opted to pool five plants for each accession. The objective of this strategy was to increase the chances to capture all the diversity available at the landraces level; the downside being an increased complexity in the effort to accurately detect alleles in bulked samples. Effective methods for SNP calling in pooled samples have been developed on genotyping array platforms (Arca et al., 2021), enabling the characterization of large pools of maize landraces (Arca et al., 2023). In our case, we used sequencing read counts for each allele at a given genomic position factoring in base quality, mapping quality, and read depth to weigh on the representativeness of each marker using VarScan (Koboldt et al., 2009). Additionally, we computed within-pool MAF finding that on average, heterozygous allele calls had a one-to-five ration of polymorphism. For each sample and marker, we cannot determine whether the MAF is contributed by heterozygous individuals or rather by a mix of different homozygote genotypes in the pool. However, we can assume that in the long range any segregating locus within a landrace will adjust to a Hardy-Weinberg equilibrium in the absence of forces of evolution. This equilibrium will be broken for those loci that are under selection for local adaptation to either agronomic performance and/or farmer preference.

We found three genetic clusters within the Bhutanese germplasm. Most accessions in Clusters 1 and 3 were from eastern Bhutan, while Cluster 2 was mostly from southwestern Bhutan (Figure 1b). Although genetic distance based on F_{st} among these subpopulations suggests a minimal degree of genetic differentiation, we identified outlier loci among them (Figure 1c). These QTNs harbor putative genes (Dataset S6) that could be further explored. For instance, outlier loci in Cluster 2 also a QTN harbors gene models including *polyamine oxidase9* an enzyme that has been shown to impact leaf elongation and overall plant growth in maize (Alberto Rodríguez et al., 2009), *protochlorophyllide reductase1* an enzyme that promotes tocopherol (Vitamin E) accumulation in both leaves and kernels of maize (Zhan et al., 2019), and *arogenate dehydrogenase1* which is involved in kernel protein metabolism; specifically tyrosine and phenylalanine (Holding et al., 2010) and *germin-like protein26 (glp26)*, glp-like proteins play a crucial role in pathogen resistance (Mao et al., 2022). It is possible that the differentiation among genetic clusters is partially contributed by selection for loci conferring traits that are relevant for local adaptation.

The highest genetic distance was observed between two clusters in eastern Bhutan, suggesting a combination of factors driving selection. In the eastern districts of Bhutan (*Dzongkhags*), maize plays a crucial role in household food security as high slopes pose

challenges for irrigated rice farming. The difficult terrain may have likely impacted seed exchange, a phenomenon previously described in Nepal (Bajracharya et al., 2010). However, as farmers have adopted the cultivation of improved varieties, active community seed exchange programs have also been adopted (Wangmo et al., 2018). The FGDs we conducted in eastern Bhutan showed that farmers select for specific traits, and consequently impact allele frequencies on locally cultivated materials. This selection may also be influenced by the cultivation of maize in Butans' heterogenous agroecologies. In which case, farmers select for different phenotypic traits depending on the microclimate and prevailing vulnerabilities. Indeed, in the women FGD, farmers explained the rationale behind some of the preferences based on the region (Dataset S9). Some farmers prioritized plants of intermediate plant height with ear placement in the middle to withstand lodging caused by strong winds and bring convenience to daily field activities. In the high altitude, early maturing varieties were prioritized to escape frost damage. In the lower elevation areas, farmers showed a preference for varieties that could resist pests and diseases.

We then explored the genomic loci associated with quantitative traits informing farmer preferences using a small common garden experiment. Arguably, our experimental design suffers several limitations, including being run in just 1 year in one location, and may not be generalized beyond the observations done in this study. However, our characterization data still gives a peek at the agronomic diversity existing in the Bhutan allele pool and its potential for incorporation into breeding programs. We identified several putative loci associated with phenotypic traits in our experiment (Dataset S14). Among them, plant height, ear height, and earliness are highly heritable polygenic traits with crucial roles for both environmental adaptation (Abendroth et al., 2021; Sandhu & Dhillon, 2021) and yield (Peiffer et al., 2014).

Maize is particularly prone to temperature and rainfall stress (Mulungu & Ng'ombe, 2019). The extent of cultivation of maize landraces in Bhutan is shrinking in favor of improved varieties as evidenced in our FGD (results not shown), a trend equally observed across the globe (Fenzi & Couix, 2021). Maize landraces have the potential to sustainably address current and future challenges affecting production (Guzzon et al., 2021). We employed GWAS to identify genomic loci associated with climate variables. Variable bio15 ranked highly in predicting the GF model and was associated with several putative loci including a QTN on Chromosome 2 at 15.3 Mb that harbors calcium-dependent protein kinase 5, a gene that plays a crucial role in plants' response to abiotic stresses (Liu et al., 2021). For bio8, we identified an associated QTN on Chromosome 7 at 170.3 Mb harboring *Cysteine proteases8 and 9*, cysteine proteases play a major role in plant growth and development, senescence, and immunity (Li et al., 2021). We did not find any overlap between QTNs associated with bioclimatic and phenotypic traits within this study. Nonetheless, it is plausible that any QTNs/loci related to environmental adaptation might align to phenotypic traits that are selected in certain pedoclimatic conditions (Caproni et al., 2023). Indeed, several bioclimatic-associated QTNs identified in our study correspond to genomic regions identified for agronomic traits in previous studies (Mural et al., 2022). The overlapping phenotypic associated traits included several aspects of plant

performance, including flowering time, inflorescence, seed composition, and vegetative traits (Dataset S13).

GF predictive models can significantly improve the ability to detect areas that are likely to be vulnerable (Rhoné et al., 2020; Caproni et al., 2023). The distribution of maize landrace diversity we observe today may suffer under future climate scenarios. Our analysis suggests that predicted variations in patterns of rain and temperature are likely to have impacts in the districts of southwestern Bhutan; in particular, the strongest offset is predicted for *Samtse*, *Chukha*, *Dagana*, *Tsirang*, and *Sarpang*. These districts, although not in the major maize production areas as in the east, are a source of unique maize agrobiodiversity identified in Cluster 2 (Figure 2c); three genotypes sourced from these regions were among the 14 top accessions prioritized by both farmers and with agronomic trait data evaluation (Dataset S1). We believe that further germplasm collection efforts, especially in these areas should be prioritized; future breeding efforts, targeting local adaptation, will benefit the availability of a larger, comprehensive Bhutanese collection of maize diversity that today may be at risk; such germplasm wealth will cushion Bhutanese smallholder farmers against future climate scenarios in Bhutan.

4.1 | Conclusion

Overall, our findings provide a glimpse of the genetic composition of Bhutan's maize landrace germplasm which is divergent from the neighboring Indian and Nepal Himalayas. Within Bhutan, there is an indication of an existing selection process as occasioned by the sub-clustering. The landrace germplasm is relatively recent as it was collected between 2005 and 2017. Without mitigation, farm landrace diversity will continue to dwindle because of different factors including changing cropping systems amidst the climate crisis. Thus, improved strategies must be adopted to mitigate prevailing vulnerabilities by utilizing this resource.

Our study involved farmers' knowledge in selecting their preferred varieties and then using this information to filter accessions by agronomic traits. By integrating traditional knowledge and quantitative data, we were able to prioritize varieties of interest. The accessions selected could be aptly used in breeding programs with higher chances of adoption by farmers. Further integration of genomic and climatic data provides information that adds to the robustness of breeding programs particularly for climate adaptation.

AUTHOR CONTRIBUTIONS

Mario Enrico Pe, Matteo Dell'Acqua, and Tashi Yangzome designed the study and supervised the research. Asta Tamang performed experiments and data analysis. Mercy Wairimu Macharia, Mara Miculan, Leonardo Caproni, and Svenja Mager managed molecular data and performed data analysis. Leonardo Caproni and Jemal Seid Ahmed contributed to the analysis of climatic data. Mercy Wairimu Macharia drafted the manuscript, interpreted results, and produced figures with contributions by Leonardo Caproni. All authors have read and approved the manuscript.

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CONFLICT OF INTEREST STATEMENT

Authors declare no competing interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article as supplementary materials. Raw reads are available at the National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov>) under BioProject ID PRJNA825653.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The research study was a collaboration between the Scuola Superiore Sant'Anna, Italy and the National Biodiversity Center (NBC), Bhutan. The NBC has the national mandate to promote collaborative research in the field of biodiversity. NBC coordinated the farmer participatory research following the institution's guidelines. In conforming to the Code of Ethics of the International Society of Ethnobiology (ISE), all participants involved in evaluating field material were above 18 years old and provided informed verbal consent before participating in the study. They were briefed about the study objectives, procedures, and benefits. Participants were assured that the data would be used strictly for research purposes as per the NBC guidelines.

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REFERENCES

- Abendroth, L. J., Miguez, F. E., Castellano, M. J., Carter, P. R., Messina, C. D., Dixon, P. M., & Hatfield, J. L. (2021). Lengthening of maize maturity time is not a widespread climate change adaptation strategy in the US Midwest. *Global Change Biology*, 27, 2426–2440. <https://doi.org/10.1111/gcb.15565>
- Acosta-Quezada, P. G., Valladolid-Salinas, E. H., Murquincho-Chuncho, J. M., Jadán-Veriñas, E., & Ruiz-González, M. X. (2022). Heterogeneous effects of climatic conditions on Andean bean landraces and cowpeas highlight alternatives for crop management and conservation. *Scientific Reports*, 12, 6586. <https://doi.org/10.1038/s41598-022-10277-x>
- Alberto Rodríguez, A., Javier Maiale, S., Bernardina Menéndez, A., & Adolfo Ruiz, O. (2009). Polyamine oxidase activity contributes to sustain maize leaf elongation under saline stress. *Journal of Experimental Botany*, 60, 4249–4262. <https://doi.org/10.1093/jxb/erp256>
- Altinsoy, H., & Kurnaz, L. (2021). A new methodology for climate model selection and application to temperature of Europe. *Theoretical and Applied Climatology*, 145, 95–107. <https://doi.org/10.1007/s00704-021-03622-z>
- Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data*. Babraham Institute. Available online at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc>
- Arca, M., Gouesnard, B., Mary-Huard, T., Le Paslier, M.-C., Bauland, C., Combes, V., Madur, D., Charcosset, A., & Nicolas, S.-D. (2023). Genotyping of DNA pools identifies untapped landraces and genomic regions to develop next-generation varieties. *Plant Biotechnology Journal*, 21(6), 1123–1139. <https://doi.org/10.1111/pbi.14022>
- Arca, M., Mary-Huard, T., Gouesnard, B., Bérard, A., Bauland, C., Combes, V., Madur, D., Charcosset, A., & Nicolas, S. D. (2021). Deciphering the genetic diversity of landraces with high-throughput SNP genotyping of DNA bulks: Methodology and application to the maize 50k array. *Frontiers in Plant Science*, 11, 568699. <https://doi.org/10.3389/fpls.2020.568699>
- Bajo. (2004) Technical guidelines for measuring crop yields in field crops. F Crop Sect RNRRC Bajo with Tech Support RNRRC Wengkar Financ Assist RNR-ESP, CORE, MoA.
- Bajracharya, J., Rana, R. B., Gauchan, D., Sthapit, B. R., Jarvis, D. I., & Witcombe, J. R. (2010). Rice landrace diversity in Nepal. Socio-economic and ecological factors determining rice landrace diversity in three agro-ecozones of Nepal based on farm surveys. *Genetic Resources and Crop Evolution*, 57, 1013–1022. <https://doi.org/10.1007/s10722-010-9544-x>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bitocchi, E., Bellucci, E., Rau, D., Albertini, E., Rodriguez, M., Veronesi, F., Attene, G., & Nanni, L. (2015). European flint landraces grown in situ reveal adaptive introgression from modern maize. *PLoS ONE*, 10(4), e0121381. <https://doi.org/10.1371/journal.pone.0121381>
- Böhm, J., Schipprack, W., Mirdita, V., Utz, H. F., & Melchinger, A. E. (2014). Breeding potential of European flint maize landraces evaluated by their testcross performance. *Crop Science*, 54, 1665–1672. <https://doi.org/10.2135/cropsci2013.12.0837>
- Caproni, L., Lakew, B. F., Kassaw, S. A., Miculan, M., Ahmed, J. S., Grazioli, S., Kidane, Y. G., Fadda, C., Pè, M. E., & Dell'Acqua, M. (2023). The genomic and bioclimatic characterization of Ethiopian barley (*Hordeum vulgare* L.) unveils challenges and opportunities to adapt to a changing climate. *Global Change Biology*, 29, 2335–2350. <https://doi.org/10.1111/gcb.16560>
- Corrado, G., & Rao, R. (2017). Towards the genomic basis of local adaptation in landraces. *Diversity*, 9(4), 51. <https://doi.org/10.3390/d9040051>
- De Kort, H., Prunier, J. G., Ducatez, S., Honnay, O., Baguette, M., Stevens, V. M., & Blanchet, S. (2021). Life history, climate and biogeography interactively affect worldwide genetic diversity of plant and animal populations. *Nature Communications*, 12(1), 516. <https://doi.org/10.1038/s41467-021-20958-2>
- Ellis, N., Smith, S. J., & CRP. (2012). Gradient forests: Calculating importance gradients on physical predictors. *Ecology*, 93, 156–168. <https://doi.org/10.1890/11-0252.1>
- Fenzi, M., & Couix, N. (2021). Growing maize landraces in industrialized countries: From the search for seeds to the emergence of new practices and values. *International Journal of Agricultural Sustainability*, 20(3), 327–345. <https://doi.org/10.1080/14735903.2021.1933360>
- Fitzpatrick, M. C., & Keller, S. R. (2015). Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters*, 18(1), 1–16. <https://doi.org/10.1111/ele.12376>
- Fróna, D., Szenderák, J., & Harangi-Rákos, M. (2019). The challenge of feeding the world. *Sustainability*, 11(20), 5816. <https://doi.org/10.3390/su11205816>
- Grote, U., Fasse, A., Nguyen, T. T., & Erenstein, O. (2021). Food security and the dynamics of wheat and maize value chains in Africa and Asia.

- Frontiers in Sustainable Food Systems*, 4, 317. <https://doi.org/10.3389/fsufs.2020.617009>
- Grzybowski, M. W., Mural, R. V., Xu, G., Turkus, J., Yang, J., & Schnable, J. C. (2023). A common resequencing-based genetic marker dataset for global maize diversity. *The Plant Journal*, 113(6), 1109–1121. <https://doi.org/10.1111/tjp.16123>
- Guan, J., Zhang, J., Gong, D., Zhang, Z., Yu, Y., Luo, G., Somta, P., Hu, Z., Wang, S., Yuan, X., Zhang, Y., Wang, Y., Chen, Y., Laosatit, K., Chen, X., Chen, H., Sha, A., Cheng, X., Xie, H., & Wang, L. (2022). Genomic analyses of rice bean landraces reveal adaptation and yield related loci to accelerate breeding. *Nature Communications*, 13(1), 5707. <https://doi.org/10.1038/s41467-022-33515-2>
- Guzzon, F., Rios, L. W. A., Cepeda, G. M. C., et al. (2021). Conservation and use of latin american maize diversity: Pillar of nutrition security and cultural heritage of humanity. *Agronomy*, 11(1), 172. <https://doi.org/10.3390/agronomy11010172>
- Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz-Sabater, J., Nicolas, J., Peubey, C., Radu, R., Schepers, D., Simmons, A., Soci, C., Abdalla, S., Abellan, X., Balsamo, G., Bechtold, P., Biavati, G., Bidlot, J., Bonavita, M., ... Thépaut, J. N. (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal Meteorological Society*, 146, 1999–2049. <https://doi.org/10.1002/qj.3803>
- Hijmans, R. J. (2015). Raster geographic data analysis and modeling. CRAN. R package version 2.4-15. <http://CRAN.R-project.org/package=raster>
- Hijmans, R. J., Phillips, S., Leathwick, J., & Elith, J. (2015). Dismo species distribution modeling. CRAN. R package version 1.0-12. <https://cran.r-project.org/web/packages/dismo/index.html>
- Holding, D. R., Meeley, R. B., Hazebroek, J., Selinger, D., Gruis, F., Jung, R., & Larkins, B. A. (2010). Identification and characterization of the maize arogenate dehydrogenase gene family. *Journal of Experimental Botany*, 61, 3663–3673. <https://doi.org/10.1093/jxb/erq179>
- Jägermeyr, J., Müller, C., Ruane, A. C., Elliott, J., Balkovic, J., Castillo, O., Faye, B., Foster, I., Folberth, C., Franke, J. A., Fuchs, K., Guarin, J. R., Heinke, J., Hoogenboom, G., Iizumi, T., Jain, A. K., Kelly, D., Khabarov, N., Lange, S., ... Rosenzweig, C. (2021). Climate impacts on global agriculture emerge earlier in new generation of climate and crop models. *Nature Food*, 2, 873–885. <https://doi.org/10.1038/s43016-021-00400-y>
- Jarvis, A., Reuter, H. I., & Nelson EG, A. (2008). *Hole-filled seamless SRTM data V4*. International Centre for Tropical Agriculture (CIAT). Available from: <http://srtm.csi.cgiar.org>
- Johannessen, C. L., & Parker, A. Z. (1989). Maize ears sculptured in 12th and 13th century A.D. India as indicators of pre-Columbian diffusion. *Economic Botany*, 43, 164–180. <https://www.jstor.org/stable/4255150>, <https://doi.org/10.1007/BF02859857>
- Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, 11, 94. <https://doi.org/10.1186/1471-2156-11-94>
- Kandel, B. P., & Shrestha, K. (2020). Performance evaluation of maize hybrids in inner-plains of Nepal. *Heliyon*, 6(12), e05542. <https://doi.org/10.1016/j.heliyon.2020.e05542>
- Katwal, T. B., Wangchuk, D., Dorji, L., et al. (2013). Evaluation of gray leaf spot tolerant genotypes from CIMMYT in the highland maize production eco-systems of Bhutan. *Journal of Life Sciences*, 7, 443–452.
- Kindt, R., & Coe, R. (2005). Analysis of counts of trees. A manual and software for common statistical methods for ecological and biodiversity studies. In *Agroforestry Centre (ICRAF)* (Vol. 6) (pp. 71–101). World.
- Koboldt, D. C., Chen, K., Wylie, T., Larson, D. E., McLellan, M. D., Mardis, E. R., Weinstock, G. M., Wilson, R. K., & Ding, L. (2009). VarScan: Variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics*, 25(17), 2283–2285. <https://doi.org/10.1093/bioinformatics/btp373>
- Krishna, H. G., Bal, K. J., Ajaya, K., Paudel, M. N. (2020). Assessing genetic diversity of Nepalese maize landraces using multivariate analysis. In *Proceedings of the 29th National Summer Crops Workshop, Lumle, Pokhara, Nepal, 16–18 June 2018*. Nepal Agricultural Research Council.
- Li, Y., Guan, K., Schnitkey, G. D., DeLucia, E., & Peng, B. (2019). Excessive rainfall leads to maize yield loss of a comparable magnitude to extreme drought in the United States. *Global Change Biology*, 25, 2325–2337. <https://doi.org/10.1111/gcb.14628>
- Li, Y., Niu, L., Wu, X., Faleri, C., Tai, F., Zhang, M., Liu, H., Wang, W., & Cai, G. (2021). Genome-wide identification and comparison of cysteine proteases in the pollen coat and other tissues in maize. *Frontiers in Plant Science*, 12, 709534. <https://doi.org/10.3389/fpls.2021.709534>
- Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., Gore, M. A., Buckler, E. S., & Zhang, Z. (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, 28, 2397–2399. <https://doi.org/10.1093/bioinformatics/bts444>
- Liu, L., Xiang, Y., Yan, J., di, P., Li, J., Sun, X., Han, G., Ni, L., Jiang, M., Yuan, J., & Zhang, A. (2021). BRASSINOSTEROID-SIGNALING KINASE 1 phosphorylating CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE functions in drought tolerance in maize. *The New Phytologist*, 231(2), 695–712. <https://doi.org/10.1111/nph.17403>
- Liu, X., Huang, M., Fan, B., Buckler, E. S., & Zhang, Z. (2016). Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genetics*, 12(2), e1005767. <https://doi.org/10.1371/journal.pgen.1005767>
- Mao, L., Ge, L., Ye, X., Xu, L., Si, W., & Ding, T. (2022). ZmGLP1, a germin-like protein from maize, plays an important role in the regulation of pathogen resistance. *International Journal of Molecular Sciences*, 23(22), 14316. <https://doi.org/10.3390/ijms232214316>
- McLean-Rodríguez, F. D., Costich, D. E., Camacho-Villa, T. C., Pè, M.-E., & Dell'Acqua, M. (2021). Genetic diversity and selection signatures in maize landraces compared across 50 years of in situ and ex situ conservation. *Heredity*, 126, 913–928. <https://doi.org/10.1038/s41437-021-00423-y>
- Meinert, C., & Sørensen, H. (2020). From Padmasambhava to Gö Tsangpa: Rethinking religious patronage in the Indian Himalayas between the 8th and 13th centuries. In *Buddhism in Central Asia I* (pp. 151–188). Brill. https://doi.org/10.1163/9789004417731_008
- Mendiburu, F. D. (2019). *Agricolae: Statistical procedures for agricultural research*. CRAN. R package version 1.3-2.
- Mir, C., Zerjal, T., Combes, V., Dumas, F., Madur, D., Bedoya, C., Dreisigacker, S., Franco, J., Grudloyma, P., Hao, P. X., Hearne, S., Jampatong, C., Laloë, D., Muthamia, Z., Nguyen, T., Prasanna, B. M., Taba, S., Xie, C. X., Yunus, M., ... Charcosset, A. (2013). Out of America: Tracing the genetic footprints of the global diffusion of maize. *Theoretical and Applied Genetics*, 126, 2671–2682. <https://doi.org/10.1007/s00122-013-2164-z>
- Mukerjee, P., Sogani, R., Gurung, N., Rastogi, A., & Swiderska, K. (2018). *Smallholder farming systems in the Indian Himalayas key trends and innovations for resilience*. IIED Country Report.
- Mulungu, K., & Ng'ombe, J. N. (2019). Climate change impacts on sustainable maize production in Sub-Saharan Africa: A review. In A. Hossain (Ed.), *Maize*. IntechOpen. <https://doi.org/10.5772/intechopen.90033>
- Mural, R. V., Sun, G., Grzybowski, M., Tross, M. C., Jin, H., Smith, C., Newton, L., Andorf, C. M., Woodhouse, M. R., Thompson, A. M., Sigmon, B., & Schnable, J. C. (2022). Association mapping across a multitude of traits collected in diverse environments in maize. *Giga-Science*, 11, giac080. <https://doi.org/10.1093/gigascience/giac080>
- Omoyo, N. N., Wakhungu, J., & Otengi, S. (2015). Effects of climate variability on maize yield in the arid and semi arid lands of lower eastern Kenya. *Agriculture & Food Security*, 4(8), 1–13. <https://doi.org/10.1186/s40066-015-0028-2>
- Peiffer, J. A., Romay, M. C., Gore, M. A., Flint-Garcia, S. A., Zhang, Z., Millard, M. J., Gardner, C. A. C., McMullen, M. D., Holland, J. B., Bradbury, P. J., & Buckler, E. S. (2014). The genetic architecture of

- maize height. *Genetics*, 196(4), 1337–1356. <https://doi.org/10.1534/genetics.113.159152>
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, 7(5), e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Petropoulos, S. A., Barros, L., & Ferreira, I. C. F. R. (2019). Editorial: Rediscovering local landraces: Shaping horticulture for the future. *Frontiers in Plant Science*, 10, 126. <https://doi.org/10.3389/fpls.2019.00126>
- Prasanna, B. (2010). Phenotypic and molecular diversity of maize landraces: Characterization and utilization. *Indian Journal of Genetics*, 70(4), 315–327.
- R Core Team. (2021). *The R project for statistical computing*. CRAN. Retrieved February 2, 2023, from <https://www.r-project.org/>
- Ranum, P., Peña-Rosas, J. P., Garcia-Casal, M. N. (2014). Global maize production, utilization, and consumption. *Annals of the new York Academy of Sciences*, 1312, 105–112. <https://doi.org/10.1111/nyas.12396>
- Rhoné, B., Defrance, D., Berthouly-Salazar, C., Mariac, C., Cubry, P., Couderc, M., Dequinsey, A., Assoumanne, A., Kane, N. A., Sultan, B., Barnaud, A., & Vigouroux, Y. (2020). Pearl millet genomic vulnerability to climate change in West Africa highlights the need for regional collaboration. *Nature Communications*, 11(1), 5274. <https://doi.org/10.1038/s41467-020-19066-4>
- Ruane, A. C., & McDermid, S. P. (2017). Selection of a representative subset of global climate models that captures the profile of regional changes for integrated climate impacts assessment. *Earth Perspectives*, 4, 1–20. <https://doi.org/10.1186/s40322-017-0036-4>
- Sanderson, B. M., Knutti, R., & Caldwell, P. (2015). A representative democracy to reduce interdependency in a multimodel ensemble. *Journal of Climate*, 28, 5171–5194. <https://doi.org/10.1175/jcli-d-14-00362.1>
- Sandhu, S., & Dhillon, B. S. (2021). Breeding plant type for adaptation to high plant density in tropical maize—A step towards productivity enhancement. *Plant Breeding*, 140, 509–518. <https://doi.org/10.1111/pbr.12949>
- Schluter, D., & Conte, G. L. (2009). Genetics and ecological speciation. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 9955–9962. <https://doi.org/10.1073/pnas.0901264106>
- Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, 3, 307–327. <https://doi.org/10.1007/s12571-011-0140-5>
- Storey, J. D. (2003). The positive false discovery rate: A Bayesian interpretation and the q-value. *The Annals of Statistics*, 31, 2013–2035. <https://doi.org/10.1214/aos/1074290335>
- Storey, J. D., & Tibshirani, R. (2003). Statistical significance for genome-wide studies. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9440–9445. <https://doi.org/10.1073/pnas.1530509100>
- Strigens, A., Schipprack, W., Reif, J. C., & Melchinger, A. E. (2013). Unlocking the genetic diversity of maize landraces with doubled haploids opens new avenues for breeding. *PLoS ONE*, 8, e57234. <https://doi.org/10.1371/journal.pone.0057234>
- Taiyun Wei, M., Taiyun Wei Cre, A., Simko Aut, V., et al. (2021). Visualization of a correlation matrix. Version 0.92. GitHub. <https://github.com/taiyun/corrplot>
- Tenaillon, M. I., & Charcosset, A. (2011). A European perspective on maize history. *Comptes Rendus Biologies*, 334(3), 221–228. <https://doi.org/10.1016/j.crvi.2010.12.015>
- Thapa, J. K. (1966). Primitive maize with the lepchas. *Bulletin of Tibetology*, 3(1), 29–31.
- Turner, S. D. (2018). QQman: An R package for visualizing GWAS results using Q-Q and Manhattan plots. *The Journal of Open Source Software*, 3(25), 731. <https://doi.org/10.21105/joss.00731>
- Uchibayashi, M. (2005). Maize in pre-Columbian China. *Yakugaku Zasshi*, 125(7), 583–586. <https://doi.org/10.1248/yakushi.125.583> PMID: 15997215
- Wangmo, P., Tashi, S., Dorji, L., Wangchuk, D., Wangdi, N., & Gyeltshen, T. (2018). Assessment of community-based maize seed production in eastern Bhutan. *Bhutanese Journal of Agriculture*, 1, 77–91.
- Weir, B. S., & Cockerham, C. C. (1984). Estimating f-statistics for the analysis of population structure. *Evolution*, 38(6), 1358–1370. <https://doi.org/10.1111/j.1558-5646.1984.tb05657.x>
- Whitlock, M. C., & Lotterhos, K. E. (2015). Reliable detection of loci responsible for local adaptation: Inference of a null model through trimming the distribution of F (ST). *The American Naturalist*, 186(Suppl 1), S24–S36. <https://doi.org/10.1086/682949>
- Wickham, H. (2016). *GGplot2: Elegant graphics for data analysis*. Springer. <https://doi.org/10.1007/978-3-319-24277-4>
- Woldeyohannes, A. B., Iohannes, S. D., Miculan, M., Caproni, L., Ahmed, J. S., de Sousa, K., Desta, E. A., Fadda, C., Pè, M. E., & Dell'Acqua, M. (2022). Data-driven, participatory characterization of traditional farmer varieties discloses teff (*Eragrostis tef*) adaptive and breeding potential under current and future climates. *eLife*, 11, e80009. <https://doi.org/10.7554/eLife.80009>
- Yang, N., Wang, Y., Liu, X., Jin, M., Vallebuena-Estrada, M., Calfee, E., Chen, L., Dilkes, B. P., Gui, S., Fan, X., Harper, T. K., Kennett, D. J., Li, W., Lu, Y., Ding, J., Chen, Z., Luo, J., Mambakkam, S., Menon, M., ... Ross-Ibarra, J. (2023). Two teosintes made modern maize. *Science*, 382, eadg8940. <https://doi.org/10.1126/science.adg8940>
- Zhan, W., Liu, J., Pan, Q., Wang, H., Yan, S., Li, K., Deng, M., Li, W., Liu, N., Kong, Q., Fernie, A. R., & Yan, J. (2019). An allele of ZmPORB2 encoding a protochlorophyllide oxidoreductase promotes tocopherol accumulation in both leaves and kernels of maize. *The Plant Journal*, 100, 114–127. <https://doi.org/10.1111/tbj.14432>
- Zhang, Z., Wei, J., Li, J., Jia, Y., Wang, W., Li, J., Lei, Z., & Gao, M. (2022). The impact of climate change on maize production: Empirical findings and implications for sustainable agricultural development. *Frontiers in Environmental Science*, 10, 1633. <https://doi.org/10.3389/fenvs.2022.954940>

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