

From Local Heritage to Genomic Innovation: Whole-Genome Sequencing of Tunisian Durum Wheat Landraces Chili and Mahmoudi

DurumGPT (Durum wheat Genome Project Tunisia) consortium¹

Abstract

Durum wheat is a cornerstone cereal of the Mediterranean region and a critical contributor to global food security, particularly under accelerating climate change. Although the crop exhibits remarkable phenotypic diversity across the basin, its genomic diversity, especially within traditional landraces, remains underexplored. Tunisia harbors a rich heritage of ancient durum wheat varieties uniquely adapted to local environments. Here, we report the whole-genome sequences of two emblematic Tunisian landraces, Mahmoudi (Arabic: محمّودي) and Chili (Arabic: شلي/شيلي). Selected over generations by farmers, these landraces combine resilience to biotic and abiotic stresses with strong adaptation to Southern Mediterranean climates. Beyond their agronomic value, Mahmoudi and Chili are deeply embedded in culinary traditions and regional identity, supporting smallholder livelihoods through exceptional grain quality, short supply chains, and artisanal processing. We anticipate that these genome sequences will catalyze research at a time when local landraces, largely displaced since the early twentieth century by high-yielding modern cultivars which suffer substantial genetic erosion, are now confined mainly to marginal farming systems. Renewed interest in climate-resilient crops highlights their potential as strategic resources for future durum wheat breeding. To accelerate discovery, we launched the Durum Genome Project Tunisia (DurumGPT), an open-science consortium dedicated to sharing genomic data, fostering collaboration, and raising awareness of Tunisia's genetic heritage. We release these whole-genome datasets without restriction through the OpenDurumGPT Community (<https://zenodo.org/communities/opendurumgpt/>). We actively encourage analyses, feedback, and contributions from the community.

Introduction

Durum wheat (*Triticum turgidum* var. *durum* Desf.) is an allotetraploid species ($2n = 4x = 28$; AABB genomes) that arose through hybridization between *Triticum urartu* and a close relative of *Aegilops speltoides* (Oliveira et al., 2012). The polyploid wild progenitor that resulted from this event persisted for several hundred thousand years before being domesticated approximately 10,000 years ago in the Fertile Crescent (Kabbaj et al., 2017; Moragues et al., 2007). Today, it ranks among the world's most widely cultivated cereals, accounting for roughly 6.9% of global wheat production across 13.7 million hectares and serving as a staple food for nearly half a billion people.

¹ Members of DurumGPT and affiliations (page 11)

The Mediterranean basin is a major center of durum wheat diversification (Bonjean, 2016; Ouaja et al., 2021; Royo et al., 2014; Soriano et al., 2018) and Tunisia contributes significantly through its extensive collection of traditional germplasms (Robbana et al., 2019; Ouaja et al., 2021). These landraces display high morphological variability shaped by long-term evolution and multigenerational farmer selection, forming a valuable reservoir for crop improvement and climate adaptation (Ayed et al., 2010; Slim et al., 2010; Krima et al., 2020, Robbana et al., 2021).

A pronounced geographic stratification characterizes Tunisian cultivation systems: modern cultivars dominate the more favorable northern regions, whereas drought-adapted landraces persist in southern drylands. However, as elsewhere, the widespread adoption of genetically uniform semi-dwarf varieties has driven substantial genetic erosion (Gharbi et al. 2000; Ammar et al. 2011). Today, landraces occupy only an estimated 1–5% of national durum wheat production (Slim et al., 2019). Many of these genetic resources are conserved *ex situ* at the National Gene Bank of Tunisia, complemented by on-farm conservation strategies that empower farmers as stewards of agrobiodiversity (Kourda et al., 2025). This dual approach strengthens agricultural resilience, preserves traditional knowledge, and supports niche markets (Varia et al., 2021). The adaptive potential of Tunisian landraces is particularly significant given climate projections for the region, including an estimated 2.1°C increase in temperature and a 20% decline in annual rainfall by 2050 (INDC, 2015; Meddi and Eslamian, 2021). Such shifts are expected to reduce soil moisture, carbon storage, and crop productivity, with global wheat yields projected to decline by approximately 4–6% per degree of warming (Arora, 2019; Anderson et al., 2020).

Beyond their agronomic value, these landraces represent a long-standing component of cereal-based farming systems and a key element of culinary and social heritage. They also provide economic opportunities for smallholder farmers through short supply chains and quality labels (e.g. terroir-based, organic, and farmers' seed systems), fostering local value creation. Pilot initiatives in several regions have further demonstrated their potential through the development of artisanal value chains based on processed products.

Mahmoudi and Chili are among the most emblematic ancient durum wheat varieties in Tunisia

To date, around 40 old durum wheat landraces are known in Tunisia through morphological characterization and classification (Ouaja et al. 2021; Deghais et al. 2007). The most well-known durum landraces are Biskri, Bidi, Mahmoudi, Chili and Jenah Khotifa. Chili and Mahmoudi (Figure 1) are among the most appreciated landraces in Tunisia and the Mediterranean basin, combining remarkable grain quality, genetic value, and strong agro-ecological adaptation. Both landraces have been widely grown for decades—Mahmoudi throughout most Tunisian regions, especially in the south and Chili mainly in central and northern Tunisia—and are still conserved today by a limited number of traditional farmers (Galtier, 2018; Slow food Presidia, 2022).

High throughput genotyping of 43 durum wheat accessions using the 25K SNP wheat array identified 11,746 high quality SNPs, enabling the construction of a phylogenetic tree with three main clusters, one of which includes the landraces Mahmoudi and Chili in closely related subclades (Miazzi et al., 2022).

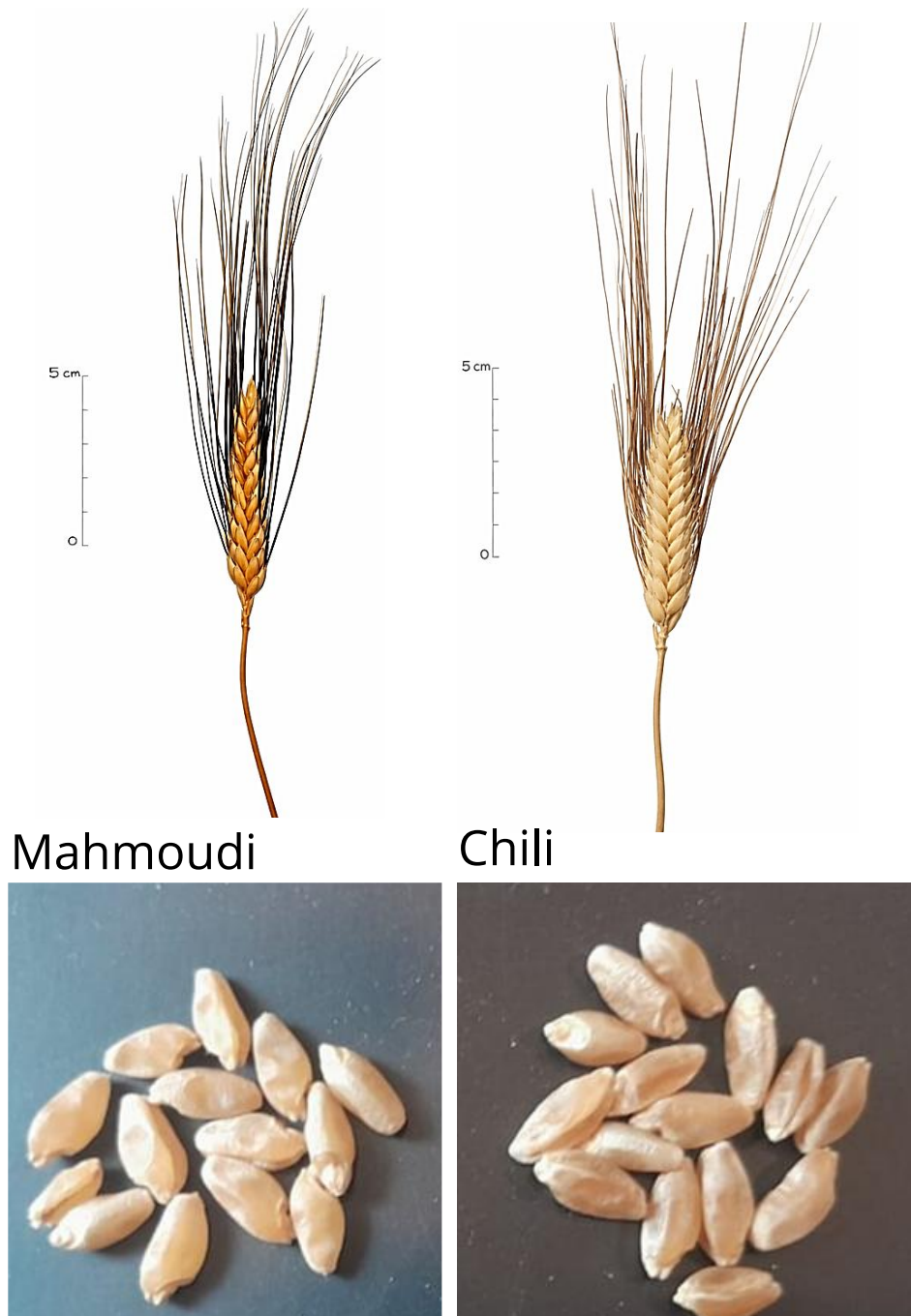


Figure 1: Spikes and grains of Chili and Mahmoudi Landraces

Some accessions being identified as synonymous, highlighting their close genetic relationship. Chili shows a slightly closer affinity to modern cultivars, consistent with its introduction from France in the early 1930s and its relatively stable yield and reduced plant height. Mahmoudi, by contrast, is a heterogeneous landrace population of multiple origins, but recognizable by particularly large spikes with sub-pyramidal shape, very long awns and large grains (Ouaja et al. 2021). Considered as one of the most ancient cultivated Tunisian durum wheat landraces, Mahmoudi is historically grown in central and southern Tunisia, especially in Kairouan, Sidi Bouzid, Kasserine, Gabès, Medenine and Gafsa (Figure 2). This landrace was historically selected for its high grain and straw yield and its outstanding tolerance to drought and heat stress, particularly in southern

regions (Othmani et al., 2019, Mangini 2022). Studies based on SSR and SNP markers revealed that it was widely distributed across different geographical locations (Ouaja et al. 2021). Mahmoudi has long been recognized as a flagship durum wheat variety by several agricultural organizations, notably the first women's agricultural development group created in Tunisia (GFDA-Oued Sbaihya-Zaghouan) and the local cooperative Lella Kmar Baya, which valorizes this landrace through its processing into semolina, flour, chorba (small pasta used in soups), and bread (Kourda et al., 2025; Inkyfada, 2023). According to Anwer Ayari, production manager and treasurer of Lella Kmar, since its creation in 2019, the cooperative has continued to thrive with a total plant area of 130 ha for cultivating Mahmoudi under conventional or organic farming. In 2025, a total of 50 tons were processed into various dietary products (Figures 3) which are destined mainly to export. The cooperative owes its success to its partnership with 20 local farmers from different regions (Siliana, Béja, Le Kef, Zaghouan, Kasserine, Sousse, Mahdia and Sidi Bouzid). For many consumers, semolina made from Mahmoudi evokes the characteristic flavor of traditional couscous prepared by their grandmothers.

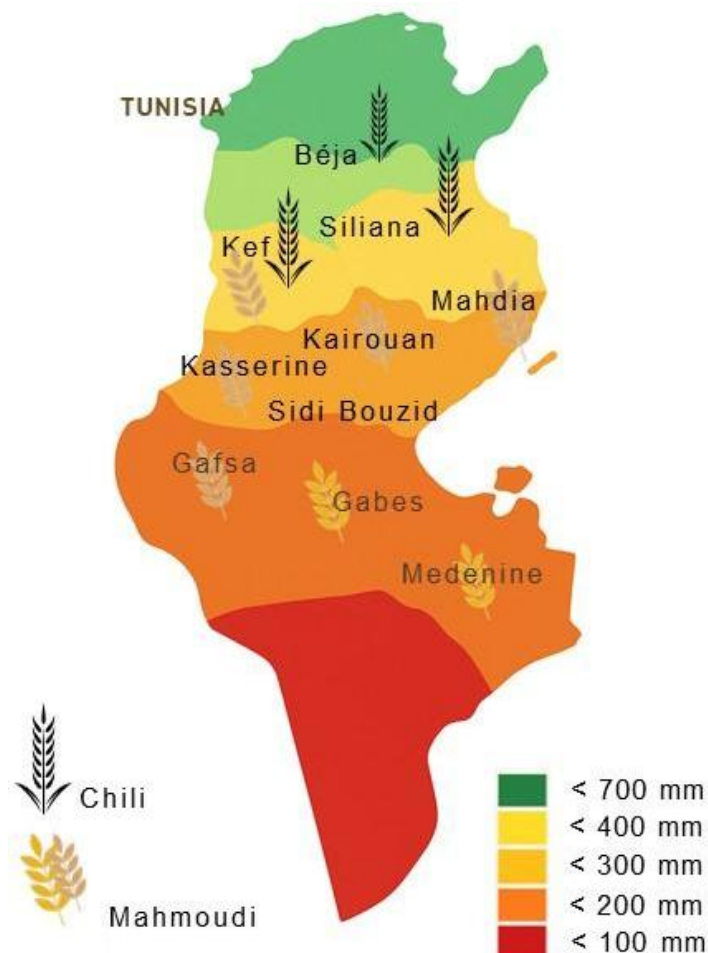


Figure 2: Geographic distribution of the cultivation's areas for Mahmoudi and Chili in Tunisia.

The Chili variety is one of Tunisia's oldest wheat landraces. It was introduced in the 1930s in Bou Salem by French farmer Charles Fabre, was officially registered in 1953, and was removed from the national catalogue in 1993 after being abandoned by large-scale farming in 1985 (Ammar et al., 2011; Deghais & al. 2007, Saade, 1996). This landrace was historically cultivated in northern and north-western Tunisia (Beja, Kef, Siliana), particularly in small plots in hilly areas or on slopes not easily accessible to mechanical

means. However, today, it is mainly cultivated by smallholder farmers engaged in organic agriculture or in farming systems with a high potential for organic conversion. Compared with modern varieties (Ben Krifa et al., 2020), Chili displays much taller stems which historically were woven into mats used to line the walls of underground pits designated for grain storage (<https://www.fondazione Slow Food.com/en/ark-of-taste-slow-food/chili-wheat/>). Beyond its agronomic performance, this variety is locally processed and valorized through traditional local knowledge, contributing to the maintenance of culturally embedded practices that strengthen its socio-economic and agri-food relevance (Kourda et al., 2025).



Figure 3: Activities of the Cooperative Lella Kmar for the Mahmoudi culture and valorisation. Key statistics for 2025 along with pictures illustrating the main products derived from Mahmoudi grains (left) and harvesting day (right) at Jof (Zaghouane, 2021). All data are courtesy of the Cooperative Lella Kmar.

From a technological perspective, Chili and Mahmoudi have a distinguished grain quality with high protein content, which refers to storage proteins such as albumins, globulins, gliadins, and glutenins in the wheat that influence nutritional value and baking properties

(Trad et al., 2014). The grain protein contents in both landraces are among the highest recorded within collections of ancient and improved durum wheats, confirming that late-maturing local varieties generally outperform early modern elite cultivars for this trait (Barneix, 2007). For instance, Mahmoudi and Chili harbor the highest semolina protein contents with 17.06% and 17.32%, respectively. Both genotypes showed similar accumulation of albumin and globulin (27% and 25%, respectively) while the total starch is about 62% (Boukid et al., 2018). Similarly, wet and dry gluten contents are significantly higher in these two landraces, compared to other improved genotypes. These different characteristics make the grains of the two landraces highly economically valuable, and their products could be promoted in national and international markets.

The two landraces exhibit higher tolerance to abiotic and biotic stresses

Mahmoudi and Chili are distinguished by their historical adaptation to Tunisian pedoclimatic conditions, and their resilience to both biotic and abiotic stresses. For instance, they showed great performance under drought. The adaptive traits of both landraces to drought include extensive root systems that enhance water extraction, improved osmotic adjustment capacity, and strategic stomatal regulation that balances carbon fixation with water conservation, as well as stable grain filling under water-limited conditions (Chaouachi et al., 2023; Chaouachi et al., 2024; Ayed et al., 2021; Othmani et al., 2019, Robbana et al., 2021)). It is worth noting that Mahmoudi has been extensively used by INRAT as a donor of drought tolerance and grain quality, and as a parent in early post-independence breeding programs (1960s–1980s). Several Tunisian cultivars trace part of their pedigree to Mahmoudi or Mahmoudi-derived selections. At the International level, Mahmoudi is conserved in several genebanks such as those at ICARDA (International Center for Agricultural Research in the Dry Areas) and CIMMYT (International Maize and Wheat Improvement Center). It has been used in pre-breeding programs as a source of abiotic stress tolerance and local Mediterranean adaptation. Moreover, Tunisian durum wheat landraces have accumulated unique combinations of resistance genes through centuries of co-evolution with local pathogens making them valuable sources of biotic stress resistance (Ferjaoui et al., 2015; 2022; Ouaja et al., 2020; 2023; 2025; Ben M'Barek & Ghaffary, 2021; Ben M'Barek et al., 2022; Laribi et al., 2022; 2023; Abdedayem et al., 2025). For septoria tritici blotch disease (STB), studies based on single-isolate inoculations revealed a wide range of responses within the landrace Mahmoudi, from resistant to intermediate and susceptible, with accessions distributed across different regions of Tunisia and no clear association between resistance level and geographical origin (Ouaja et al., 2020). In line with this variability, Ben Krime et al. (2020) demonstrated that Mahmoudi is a genetically and phenotypically heterogeneous landrace, exhibiting varying levels of quantitative resistance to STB rather than uniform resistance. In contrast, field evaluations using bulk pathogen populations showed that Mahmoudi populations generally displayed good to high levels of resistance, suggesting their potential exploitation in Mediterranean environments, whereas Chili was classified as moderately resistant (Ben M'Barek et al., 2022). This significant intra-landrace diversity is a defining trait of traditional germplasm, indicating that Mahmoudi serves as a crucial reservoir of quantitative resistance to STB.

Beyond foliar diseases, Mahmoudi represents a valuable source of resistance to emerging stem rust (*Puccinia graminis* f. sp. *tritici*), including Mediterranean-specific races such as TTRTF, a Sicilian race that caused a major outbreak in Italy in 2016. Notably, some Mahmoudi accessions also showed resistance to the Ug99 (TTKSK) race, one of the most threatening stem rust races worldwide, demonstrating that both major-gene and partial resistance mechanisms coexist within Tunisian durum wheat germplasm (Abdedayem et al., 2025).

Mahmoudi and Chili as pillars of the Durum Genome Project Tunisia

Given their high nutritional value and notable resilience to abiotic and biotic stresses, and to leverage their genetic diversity, we initiated the Durum Genome Project Tunisia (DurumGPT) by generating a comprehensive genomic resource comprising whole-genome sequencing, transcriptomics and proximity ligation data for the two durum wheat landraces Mahmoudi and Chili. We employed multiple sequencing strategies including PacBio HiFi, Illumina short-read sequencing, RNA sequencing and LinkPrep™ library preparation and sequencing. These data have been made available via NCBI/GenBank under BioProject PRJNA1420514 (details in Table 3).

The PacBio HiFi sequencing data for the Chili and Mahmoudi samples showed long reads of suitable quality, with mean read lengths between ~10 kb and ~13.6 kb and maximum read lengths exceeding 50 kb, falling within the expected range for HiFi libraries. Mahmoudi exhibits slightly longer and more uniform reads (N50 ~14.6 kb) compared to Chili (~11.7 kb), which will support more continuous assembly. Total yields are 160 Gb for Chili and 199 Gb for Mahmoudi, confirming successful sequencing runs. Coverage of the highly repetitive 10.4 Gb tetraploid wheat genome corresponds to ~15× and ~19×, respectively which is unlikely to generate non-collapsed assemblies of the durum wheat A and B subgenomes.

Table1. PacBio HiFi CCS sequencing yield and read statistics.

	Total bases (Gb)	Read count	Mean read length (bp)	N50 read length (bp)	Max read length (bp)
Chili	160.1	15,974,893	10,023	11,676	52,327
Mahmoudi	198.6	14,620,562	13,585	14,571	54,731

Illumina paired-end 150bp-sequencing was performed across five lines for each Mahmoudi and Chili, generating between 392.1 Gb (Mahmoudi) and 440.4 Gb (Chili) of high-quality data. Raw reads yielded to a high proportion of effective clean reads (98.7–99.1%) with a consistently low estimated error rate of 0.01%. Q30 scores ranged from 97.3% to 97.8%, suitable for k-mer analysis and variant detection. GC content remained stable and within the expected range for wheat (45.8–46.2%).

By generating chromatin proximity ligation (LinkPrep™: Dovetail Genomics, USA) sequencing data, we aim to support assembly and scaffolding. Two libraries were generated for Chili and a single library for Mahmoudi and sequenced via the Illumina NovaSeq X Plus platform. The data generated range between 638,183,176 raw reads (95.7 Gb) and 661,479,414 raw reads (99.2 Gb) with Q30 values ranging from 92.90% to 93.01% (Table 2).

Table 2: LinkPrep proximity ligation sequencing yield and quality metrics.

	Raw reads	Raw data	Q20	Q30	Error rate	%GC
Chili#1	661,479,414	99.2 Gb	98.12%	93.01%	0.01%	46.47%
Chili#2	638,183,176	95.7 Gb	98.14%	92.95%	0.01%	46.33%
Mahmoudi	642,129,678	96.3 Gb	98.15%	92.90%	0.01%	46.70%

Root and shoot tissues from both landraces were harvested for RNA sequencing. The data comprises about 61–73 million raw reads, corresponding to ~9.3–10.9 Gb of data per library, with very high proportions of effective clean reads (97.8–98.9%) after filtering. The Q30 values are between 97.24% and 97.41%, indicating low error rates (0.01%) and strong suitability for transcriptome assembly, expression analysis, and gene annotation. GC content ranges from 51.3% to 54.0%, which is typical for wheat transcriptomes and shows no signs of bias or degradation.

Table 3: Accession numbers for raw sequencing data deposited at NCBI/GenBank.

Bioproject	BioSample		WGS PacBio HiFi	WGS Illumina short-read	WGS- Illumina Proximity ligation	RNA-Seq
PRJNA1420514	Chili	SAMN55233146	SRR37168548	SRR37204970 SRR37204971 SRR37204972 SRR37204973 SRR37204974	SRR37442381 SRR37421014	Root: SRR37213950 Leaf: SRR37213951
	Mahmoudi	SAMN55233147	SRR37229182	SRR37252005 SRR37252004 SRR37252003 SRR37252002 SRR37252001	SRR37457508	Root: SRR37252182 Leaf: SRR37252142

Overall, the datasets appear to be of high quality and will enable assembly of both genomes with good accuracy. These data should support downstream analysis including, k-mer analyses and variant calling, estimation of the heterozygosity of both landraces and other comparative studies in durum wheat. These datasets offer a unique opportunity, supporting the scientific community with a framework to dissect the genetic diversity of these two landraces and discover genes/networks underpinning important agronomic traits in durum wheat.

Concluding remarks

The OpenDurumGPT constitutes a novel initiative to develop a comprehensive genomic resource for local durum landraces, grounded in open science principles. This effort is intended to benefit not only the Tunisian scientific community but also the global wheat community, serving as a model for the transformative potential of local empowerment and open science. The DurumGPT consortium, with the wider Tunisian scientific community will, in collaboration with pioneering experts in wheat genomics worldwide, seek to transform the data generated from Mahmoudi and Chili into a gold mine that can

be harnessed to accelerate future breeding programs for more climate resilient durum wheat varieties.

Statement on continuing work

The datasets are being shared prior to formal publication and should be considered preliminary. We encourage and welcome analyses and feedback from the wheat genomics community and beyond. Please use the Zenodo OpenDurumGPT Community (<https://zenodo.org/communities/opendurumgpt/>) to share any analysis reports or get in touch with Moez Hanin (moez.hanin@isbs.usf.tn). We will post our own analyses of these datasets as soon as completed.

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Material and Methods

Material

Seeds of Chili and Mahmoudi landraces were provided by the Tunisian National Gene Bank.

Plant growth and DNA extraction

Seeds originated from a single head were germinated grown in pots under standard growth conditions until 10 days post-germination, at which 5 g of either leaf or root tissues were harvested and flash-frozen.

For both long and short reads whole genome sequencing strategies, nuclei extraction from leaves of both landraces was performed using the method described by Jones et al. (2024) with the PacBio Nanobind Plant Nuclei Kit (PacBio). High-molecular-weight DNA quality control was performed using Femto Pulse (Agilent 5400) and DNA was quantified with dsDNA assay (Qubit).

PacBio HiFi sequencing

PacBio HiFi library was prepared using SMRTbell Template Prep Kit 3.0 (PacBio) by Novogene (Beijing, China). Library quality and fragment size distribution were assessed using a Femto Pulse system. Sequencing was performed in circular consensus sequencing (CCS) mode on the PacBio Revio platform.

Illumina short read sequencing

Illumina whole-genome short read sequencing was performed by Novogene (Beijing, China) by preparing 350 bp-libraries using a standard plant/animal whole-genome library preparation workflow (Novogene). Library quality, fragment size distribution, and purity were assessed using an Agilent 5400 system prior to sequencing. Libraries were sequenced on the Illumina NovaSeq X Plus platform to generate paired-end reads of 150 bp (PE150).

Raw sequencing reads were subjected to quality control and filtering to remove technical artefacts and low-quality data. Adapter sequences were removed, reads containing more

than 10% ambiguous bases (N) were discarded, and reads in which more than 50% of bases had a Phred quality score ≤ 5 were excluded. Quality filtering and adapter trimming were performed using Trimmomatic (v0.36), and read quality was assessed before and after filtering using FastQC (v0.11.9). Cleaned reads were retained for downstream analyses.

LinkPrep™ libraries

Proximity ligation libraries were prepared using the LinkPrep™ Kit (Dovetail Genomics, Cat. 21025G) following the manufacturer's protocol. High-molecular-weight (HMW) genomic DNA was processed for chromatin conformation capture-based proximity ligation library construction and prepared for Illumina paired-end sequencing on the Illumina NovaSeq X Plus platform.

RNA-Seq

For RNA sequencing, total RNA was extracted separately from frozen leaf and root tissues using the Monarch Total RNA Miniprep Kit (New England Biolabs), following the manufacturer's instructions for tough-to-lyse plant samples. Frozen tissues were mechanically disrupted under liquid nitrogen using a bead-based homogenization system. Samples were resuspended in 1× Monarch DNA/RNA Protection Reagent prior to lysis to preserve RNA integrity. Following mechanical homogenization, lysates were clarified by centrifugation and RNA was purified using silica spin columns. Genomic DNA was removed using on-column DNase I treatment. RNA was eluted in nuclease-free water and stored at -80°C prior to library preparation. RNA quantity, integrity, and purity were assessed as part of quality control prior to sequencing. The mRNAs were purified from total RNA using poly-T oligo-attached magnetic beads. Purified mRNAs were fragmented, and first-strand cDNA synthesis was performed using random hexamer primers, followed by second-strand cDNA synthesis. Libraries were prepared through end repair, A-tailing, adapter ligation, size selection, PCR amplification, and purification. Libraries were sequenced on the Illumina NovaSeq X Plus platform to generate paired-end reads of 150 bp. Raw reads were processed using the same quality filtering criteria applied to whole-genome sequencing data. Filtered reads were used for downstream transcriptomic analyses.

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