Víquez-Zamora *et al.*
CABI Agriculture and Bioscience (2022) 3:68
https://doi.org/10.1186/s43170-022-00135-1

CABI Agriculture and Bioscience

RESEARCH

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Diversity in mitotic DNA repair efficiencies between commercial inbred maize lines and native Central American purple landraces

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Abstract

Background: Homologous recombination allows plants to repair double strand breaks in DNA, which if unrepaired may lead to cell death. In this project, we determined the relative DNA repair efficiency of the US inbred lines B73 and Mo17 and Central American purple landraces from Guatemala and Costa Rica with the purpose to uncover genetic differences that may allow for the breeding of new lines better adapted to tolerate DNA damage caused by environmental factors.

Methods: Single cell electrophoresis was used to analyze the relative DNA repair ability of several lines from the US and Central America exposed to radiomimetic agent Zeocin, and these results were in turn compared with High Resolution Melting analyses of key genes for homologous DNA recombination. The significance of differences between treatments was evaluated with the Di Rienzo, Guzmán and Casanoves (DGC) test, while High Resolution Melting and difference curves were generated with the R package "HRM.curve". Curves were created as a negative first derivative (−d(RFU)/d(T)) using normalized relative fluorescence values (RFUs) after background removal. The kinase gene *ZeaATM1* was amplified and sequenced in B73, Mo17, P1 and P2. Multiple sequence alignment of DNA and aminoacid sequences was performed using ClustalW. Protein sequence analysis was done in UniProt to compare the resulting aminoacid sequences from maize to the available sequences from *Arabidopsis thaliana* ecotype Col-0 (ATM protein code: Q9M3G7).

Results: Single-cell electrophoresis results of statistical significance suggested that the landrace P1-Pujagua Santa Cruz is resistant to damage caused by the radiomimetic agent Zeocin, and landrace P2-Pujagua La Cruz was able to repair all DNA damage after 24 h of treatment and 1 h of recovery time. In contrast, line Mo17 was unable to repair the damage, but B73 and the landraces Jocopilas (Guatemalan), Orotina Congo, and Talamanca were partially able to repair the DNA damage. High resolution melting analysis of the putative homologous DNA repair gene *ZeaATM1* revealed that landraces P1 and P2 may harbor polymorphisms for this gene, and P1 may harbor other polymorphisms for the transcription factor *ZeaSOG1* as well as the tumor suppressor *ZeaRAD51* and recombinase *ZeaBRCA1*. The kinase gene *ZeaATM1* was sequenced, and results indicate that in lines P1 and P2 there are polymorphisms near and within the FATC domain, a domain required for the activation of ATM1-mediated repair of DNA damage.

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Conclusions: Significant differences in DNA repair efficiency exist between inbred lines and landraces of maize and may be linked to allelic diversity in key genes for homologous recombination. Thus, Central American landraces could be used for breeding tolerance to genotoxic stress.

Keywords: Maize, Biodiversity, Genotoxicity, DNA damage, Central America, Mitotic DNA damage, Zeocin

Introduction

Across the world, agricultural production needs to keep pace with a growing population. However, by the year 2050, crop production might no longer meet demand (Ray et al. 2013). Thus, plant breeding to obtain new varieties of major crops is key to boosting yields and matching future demand (Scheben and Edwards 2018).

Maize cultivation and consumption are great contributors to food security and economic progress in the developing world, especially in sub-Saharan Africa, Latin America, and Asia (Cairns and Prasanna 2018). More than 300 million metric tons of maize are produced on more than 90 million hectares across these three continents. Drought- and heat-tolerant maize varieties developed by The International Maize and Wheat Improvement Center (CIMMYT) could provide a yield increase of 5–25% (Cairns and Prasanna 2018). Thus, conventional plant breeding efforts are essential to protect maize yields in countries such as Ethiopia, Nigeria, Bangladesh, India, Nepal, Pakistan, and Mexico (Cairns and Prasanna 2018).

The maize genome (*Zea mays* ssp. *mays*) has high variability and complexity. An example of this is the difference in genome sizes among lines. For instance, the genome of the reference line B73 is 2300 Mb in length, whereas lines of tropical origin are about 2810 Mb and lines of temperate origin are about 2680 Mb (Jian et al. 2017). Despite the large genome size, only 0.6% of the maize chromatin is active and only 2% of the genome may contain loci that are protein-coding (Eli et al. 2016). Furthermore, 85% of the genome consists of transposons (Schnable et al. 2009) (i.e., repetitive sequences that can move in the genome causing mutations), which may account for about 10% of non-syntenic gene variation between lines B73 and Mo17 (Sun et al. 2018).

Breeding in maize may benefit greatly from incorporating genomic diversity via introgression of alleles from wild relatives or landraces into hybrids (Hufford et al. 2012). Archeological, isotopic, and molecular evidence suggests continuous waves of maize dispersal throughout Central and South America starting 7500 years ago. This dispersal may have enhanced interspecific admixture and diversity and led to increased productivity (Kristler et al. 2020). Evidence for maize cultivation as a staple grain in Central America is as old as 4300 BC (Kennett et al. 2017), and maize pollen samples in Guanacaste, Costa Rica date back to 3550 BC (Arford and Horn 2004; Horn 2006). However, despite the long evolutionary history of the Central American landraces and their possible diversity, they remain poorly characterized. Therefore, their usefulness is largely unexplored (Bedoya et al. 2017).

Besides increasing the severity of extreme weather (Wheeler and Braun 2013), anthropogenic climate change may lead to progressive suppression of cloud formation, thus reducing the partial filtering of shortwave UV-B radiation (290–320 nm), especially when the cloud cover is less than 50% (Diffey 2002; Lindfors and Arola 2008; Schneider et al. 2019; Németh et al. 1996). For maize, exposure to a high dose of UV-B radiation increases sensitivity to drought by reducing leaf conductance, water-use efficiency, and leaf area (Krupa and Jäger 1996). When combined with increased atmospheric CO_2, UV-B radiation decreases the maize yield even more (Wijewardana et al. 2016). In plants, UV-B radiation causes the formation of cyblobutane pyrimidine dimers (CPDs) in DNA, which lead to double-strand breaks (DSBs) that require repair by nucleotide excision repair or homologous recombination (HR) (Nisa et al. 2019; Ries et al. 2000). Also, in organisms such as *Arabidopsis*, UV-B–mediated intrachromosomal HR may rely on energy supplied by photosynthesis, and a linear relationship may exist between the concentration of CPDs and the recombination frequency (Ries et al. 2000). Removal of CPDs after exposure to UV-B radiation is mediated by 1) the type II photolyase PHOTOREACTIVATING ENZYME 1 [corresponding *uvr2-1 Arabidopsis* mutants show severe withering and necrosis (Landry et al. 1997)], and 2) the XPF endonuclease [hypocotyls of corresponding *xpf-3* and *uvr1-1* mutants do not elongate (Biever et al. 2014)]. Additionally, in tropical soils, excess aluminum (Al^{3+}) causes damage to root cells (Panhwar et al. 2015), and in the model plant *Arabidopsis thaliana*, exposure to Al^{3+} causes DSBs in DNA that require repair by HR (Sjögren and Larsen 2017). Thus, DNA recombination in maize might constitute a broad tolerance mechanism against environmental genotoxicity.

In *A. thaliana*, repair of DSBs relies on the activity of several effectors (Ko et al. 2014) such as the serine/threonine kinase ATAXIA TELANGIECTASIA MUTATED (ATM), a large protein of 350 kDa that is normally present as an inactive dimer (Kurzbauer et al. 2021). In response to DNA damage, ATM self-phosphorylates

and becomes an active monomer that phosphorylates a wide array of target proteins involved in cell cycle checkpoints and DNA repair, such as cyclins, cyclin-dependent kinases, and transcription factors such as SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1; Kurzbauer et al. 2021; Ko et al. 2014). In turn, SOG1 targets genes such as the classical tumor-suppressor gene *BREAST CANCER SUSCEPTIBILITY GENE 1* (*BRCA1*) and the recombinase gene *RADIATION SENSITIVE 51* (*RAD51*), which are required for enforcement of the G_1/S and G_2/M cell-cycle checkpoints and high-fidelity homologous recombination. SOG1 also targets *LUPUS KU AUTOANTIGEN PROTEIN P80* (*KU80*) and *DNA LIGASE 4* (*LIG4*), which are required for low-fidelity non-homologous end-joining (Dorn et al. 2019; Pfeffer et al. 2017; Schröpfer et al. 2014; Ko et al. 2014; Lim et al. 2020).

This study aimed to characterize the DNA repair efficiency in Central American maize landraces to uncover potentially useful allelic diversity that could be exploited in breeding efforts for tolerance to DSBs via HR. Our results show that Costa Rican purple landraces might represent a readily available reservoir of alleles for enhanced HR.

Materials and methods

B73 (origin: Iowa, USA) and Mo17 (origin: Missouri, USA) inbred lines were provided by Rachel Wang (Academia Sinica, Taipei) and Wojtek Pawlowski (Cornell University), respectively, and were imported with permission from the Ministry of Agriculture of Costa Rica. Central American purple landraces, Jocopilas (origin: San Pedro Jocopilas, El Quiché Department, Guatemala, code 8689) and Talamanca (origin: Limón province, Costa Rica, code 8290) were provided by Daniel Fernández at the Tropical Agricultural Research and Higher Education Center (CATIE, Turrialba, Costa Rica). Landraces P2-Pujagua La Cruz (origin: La Cruz, Guanacaste, Costa Rica), P1-Pujagua Santa Cruz (origin: Santa Cruz, Guanacaste, Costa Rica) and Orotina Congo (origin: Orotina, Alajuela, Costa Rica) were collected for research purposes with permission from the University of Costa Rica (see Table 1).

Plants were carefully grown at a dedicated greenhouse serviced by drip irrigation located at the Fabio Baudrit Agricultural Research Station, in Alajuela, Costa Rica. Female and male flowers were bagged at emergence to prevent cross pollination and were properly labeled after self-pollination for eventual harvest.

To induce DNA damage, 1-week-old seedlings, obtained from seeds harvested from the greenhouse plants, were exposed to the radiomimetic agent zeocin (Sigma-Aldrich, St. Louis, MO, USA) (100 μg^{-mL}/ on distilled water), on 90-mm Petri dishes, for 24 h in the dark, as per Meschichi et al. (2021). To determine relative recovery rates from damage by DSBs, some of the samples were collected immediately after zeocin treatment, and others were collected after being washed and allowed to rest for 1 h in water, as per Sakamoto et al. (2022). Neutral comet assay for detecting DSBs involved using the CometAssay Kit (96-well slides; Trevigen, Gaithersburg, MD, USA) on a CometAssay Electrophoresis System II, following the manufacturer's instructions and the guidelines for Minimum Information for Reporting on the Comet Assay (MIRCA) (Møller et al. 2020). Three seedlings per treatment were sampled, and 300 mg fresh weight of plant material was placed on a Petri dish filled with 2 mL of 1X phosphate-buffered saline (PBS) + 20 mM ethylenediaminetetraacetic acid

Table 1 US maize lines and Central American purple landraces used in this study, including the place of origin, latitude in the Northern Hemisphere, elevation, and direct solar irradiance over time (kWh/m^2)

Name	Place of origin	Latitude	Elevation (meters above sea level)	Solar irradiance (kWh-m^2)
B73	Iowa, USA	40.13	357	1395
Mo17	Missouri, USA	38.45	236	1679
Jocopilas 8689	Guatemala, El Quiché Department, San Pedro Jocopilas village. Highland rainforest	15.09	2107	1982
P1-Pujagua La Cruz	Costa Rica, Guanacaste Province, La Cruz township. Central American Pacific drylands	11.07	232	1741
P2-Pujagua Santa Cruz	Costa Rica, Guanacaste Province, Santa Cruz township. Central American Pacific drylands	10.27	50	1843
Orotina Congo	Costa Rica, Alajuela Province, Orotina township. Central American Pacific drylands	9.91	235	1703
Talamanca 8290	Costa Rica, Limón Province, Talamanca county. Caribbean rainforest	9.6	23	1083

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(Sigma-Aldrich). The tissue from the first true leaves was finely chopped with clean razor blades to release nuclei. The resulting nuclear solution was filtered twice through a 30 µm mesh (CellTrics-Sysmex, Germany) and 3 µg was placed in a 0.2-mL Eppendorf tube containing 10 µL of low melting agarose (Trevigen) at 37 °C. Approximately 10 µL agarose solution was placed per well, then allowed to dry in the dark for 24 h at 4 °C. Nuclei were then processed with the CometAssay Kit for electrophoresis (2 h at 1 V^{-cm}, 130 mA, at 4 °C, in the dark) and stained with 50 µl undiluted SYBR Gold (Thermo Scientific, Waltham, MA, USA) for 30 min (in the dark). Slides were examined under an Olympus BX53 fluorescence microscope (Olympus, Tokyo). At least 50 nuclei per replicate were observed and scored by using TriTek CometScore 1.5 (Sumerduck, VA, USA). Statistical analyses of tail-DNA involved the Shapiro-Wilks (for normality) and Levene (for equality of variances) tests. The significance of differences between treatments was evaluated with the Di Rienzo, Guzmán and Casanoves (DGC) test (Di Renzo et al., 2002). Analyses involved using InfoStat 2020 (University of Cordoba, Argentina, https://www.infostat.com.ar/).

To detect the presence of polymorphisms in putative genes in maize related to recovery from DNA damage by DSBs, seedlings were treated for 24 h with zeocin as described above. At least 50 mg coleoptile tissue was collected per sample, placed on liquid nitrogen, and ground by using a Geno/Grinder (SPEX Sample Prep LLC, Metuchen, NJ, USA). Genomic DNA was extracted by using the cetyl trimethylammonium bromide (CTAB) method. High-resolution melting curves were created with the GoTaq PCR MasterMix (Promega, Madison, WI, USA) on a Rotor-Gene Q machine (Qiagen, Hilden, Germany). Primers were manufactured by Macrogen (Seoul, Korea). The primer sequences were for *ZeaATM1*, forward: 5′-ACCTTACGATGGCAACAAGG-3′, reverse: 5′-CACAACCGATCAACATCCAC-3′; *ZeaSOG1*, forward: 5′-TGCACATGGCTAAGTTCCTG-3′, reverse: 5′-AATGGGCTTGAACTGTGGTC-3′; *ZeaRAD51*, forward: 5′-ATTGGAGGAAACATCATGGC-3′, reverse: 5′-ATCAACTGGAGGAGGAGCAA-3′; and *ZeaBRCA1*, forward: 5′-AAAGCCAAACCAGAAGGACA-3′, reverse: 5′-AGGTGCTTCAATGTCCAACC-3′.Primers were designed to amplify homologous genes in maize based on *Arabidopsis* coding sequences retrieved by a BLAST search of the MaizeGDB website (https://www.maizegdb.org/). Primer Express 3.0 (Thermo-Fisher) was used to design primers. The expected amplicons were 250 bp in length.

The program for PCR profiling was one activation cycle of 95 °C for 1 min, 40 cycles of denaturation at 95 °C for 5 s and annealing-extension for 60 °C for 30 s, and one final cycle of dissociation from 60–95 °C with an increase in 0.3 °C every 5 s. The resulting post-PCR high resolution melting and difference curves were generated by using the R package "HRM.curve" (Schiwek et al. 2020). Melting curves were created as a negative first derivative (−d(RFU)/d(T)) using non-normalized relative fluorescence values (RFUs) after background removal.

To map putative nucleotide polymorphisms, PCR products corresponding to *ATM* from Mo17, B73, P1 and P2 were sequenced by Macrogen Inc. (Seoul, Korea). For quality control purposes, results were manually checked to remove mismatches at the 5′and 3′ends. Multiple sequence alignment of DNA and aminoacid sequences involved using ClustalW (Thompson et al. 1994). Protein sequence analysis involved using UniProt (Wang et al. 2021) to compare the resulting aminoacid sequences from maize to the available sequences from *A. thaliana* (ATM protein code: Q9M3G7).

Results

Zeocin was previously used to induce DSBs in DNA of *A. thaliana* (Ko et al. 2014), the single-celled alga *Chlamydomonas reinhardtii* (Chankova et al. 2007), and maize (Pedrosa-Garcia et al., 2021). In the present study, zeocin at 100 μg^{-mL} for 24 h generated DSBs in all maize accessions except the Costa Rican landrace Pujagua Santa Cruz (P1). The seedlings of this landrace had the lowest percentage of DNA in the tail after 24 h of exposure (Figs. 1 and 2), so there was no DNA damage (i.e., the percentage damage was equal to that of the untreated seedlings [control]). Therefore, at the doses assayed, this landrace was the only one resistant to zeocin damage. The DNA of the US inbred line Mo17 was damaged after 24 h of zeocin treatment, and this maize line was the only one not efficient in repairing the damage after 1 h recovery. The remaining maize inbred lines or landraces were partially tolerant to the DNA damage, and some showed partial DNA repair (e.g., inbred line B73 and landraces Orotina Congo, Jocopilas and Talamanca) or full repair (e.g., landrace P2-Pujagua La Cruz) after 1 h of recovery. As suggested by Rundell et al. (2003), diffuse nuclei were not observed for cases of non-repairable nuclear fragmentation, so in maize, the neutral comet assay may reliably measure mutagen-induced DNA damage. Although data correspond to a pool of biological samples, not individual samples, statistical analyses suggested the presence of significant homogeneity within each accession (Fig. 2), thus allowing for meaningful comparisons across different accessions.

Results from our comet assay to detect DNA damage suggested four main types of DNA repair efficiencies in maize. Full apparent resistance may be associated with protective metabolites such as maysin and anthocyanins

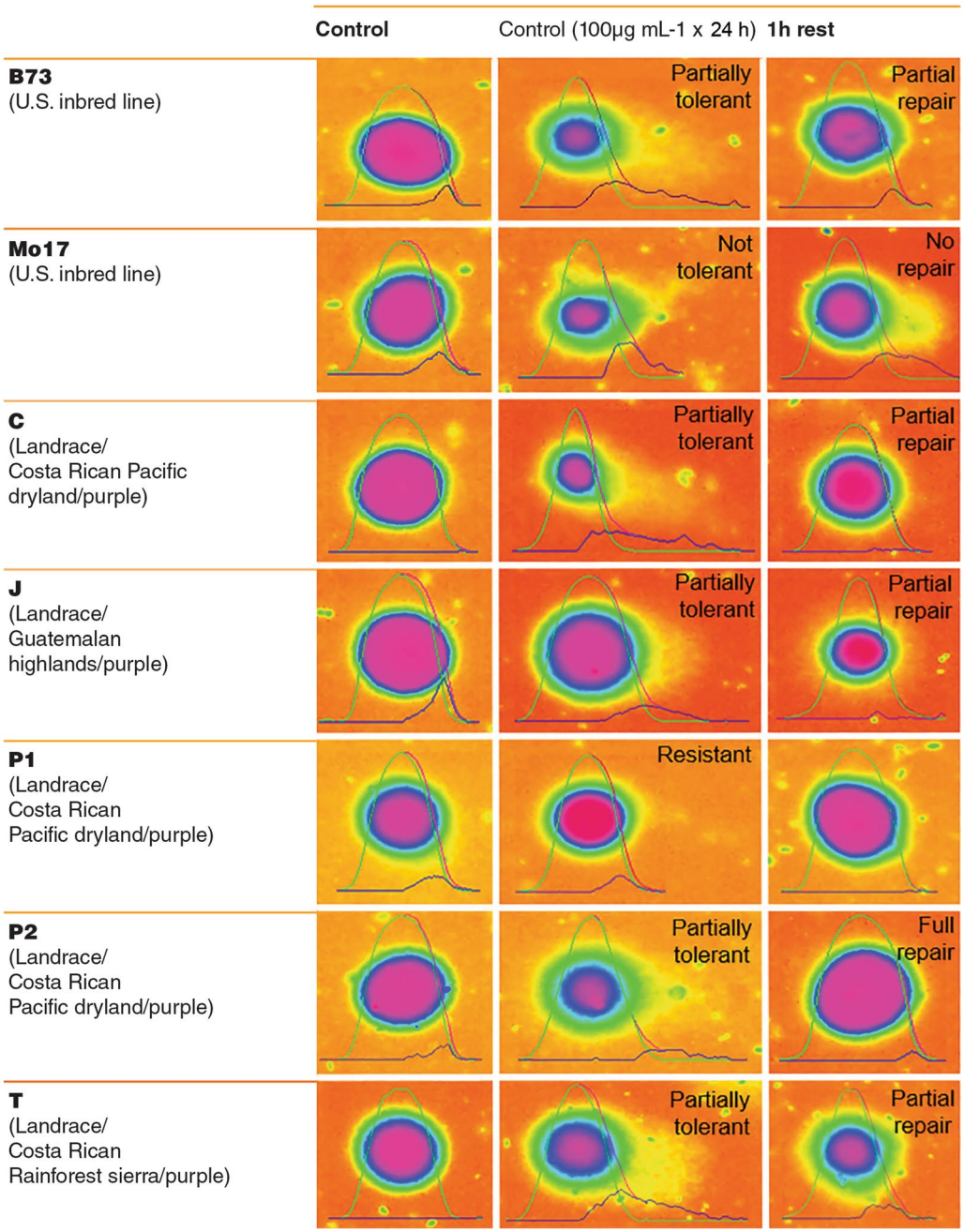

Fig. 1 Recovery from DNA damage in US maize inbred lines (B73 and Mo17) and purple Central American landraces (Orotina Congo [C], Jocopilas [J], P1-Pujagua Santa Cruz, P2-Pujagua La Cruz, Talamanca [T]) as observed by comet assay. The comet assay is a gel electrophoresis-based method that can be used to measure DNA damage in individual cells. If negatively charged DNA contains breaks, those broken ends migrate toward the anode and form a tail; thus, the amount of DNA in the tail is related to the severity of damage. In this study, seedlings were treated with zeocin (100 μg^{-mL}) for 24 h. DNA damage was compared to an untreated control. 1 h refers to samples collected after being washed and allowed to recover for 1 h in water ($n = 3$, 3 biological samples, 3 technical replicates and 50 nuclei per replicate)

(Casati and Walbot 2005) present in purple landraces, as possibly observed in landrace P1, in which no DNA tail was detected. Full repair efficiency may be related to quick and efficient repair of DSBs and may have occurred in landrace P2. Line B73 and landraces Orotina Congo, Jocopilas and Talamanca showed partial repair efficiency. Finally, the inbred line Mo17 showed limited and extremely low repair efficiency.

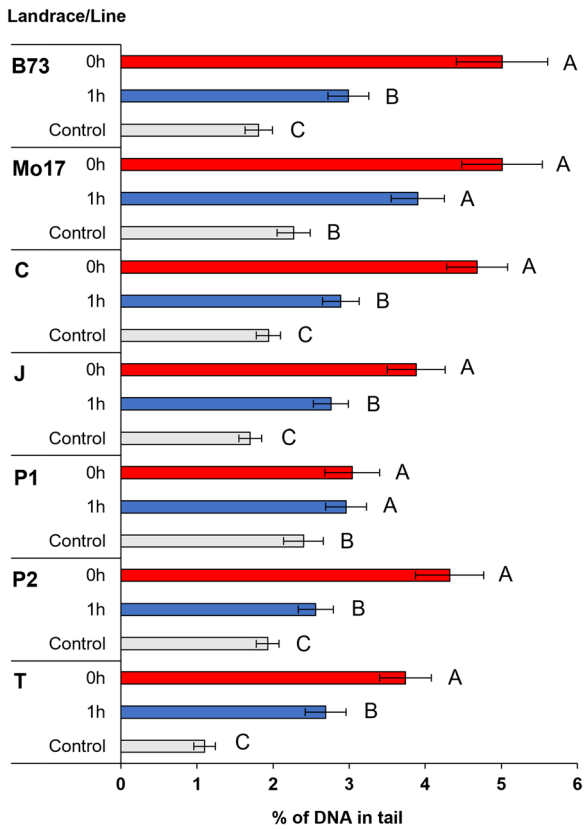

Fig. 2 DNA repair efficiency in maize inbred lines (B73 and Mo17) and purple Central American landraces (see Fig. 1 for abbreviations). DNA damage was compared to an untreated control. Data are mean ± SEM. Statistical analysis involved the Di Rienzo, Guzmán and Casanoves method. Different letters indicate significant differences across treatments within the same maize line or landrace at $P < 0.05$ ($n = 3$, with 3 biological replicates, 3 technical replicates and 50 nuclei per replicate)

For genes related to homology-dependent repair of DSBs of DNA, the putative maize homologs *ZeaATM1* (*GRMZM2G004593, ZEAMMB73_825351*), *ZeaSOG1* (*GRMZM2G027309*), *ZeaRAD51* (*Zm00001eb107510, GRMZM2G310868*) and *ZeaBRCA1* (*GRMZM2G080314*) showed clear melting peaks (Figs. 3 and 4). *ZeaATM1* and *ZeaSOG1* showed major differences in melting temperatures between the inbred lines (B73 and Mo17) and the Costa Rican landraces P1 and P2, which may suggest the presence of polymorphisms (Fig. 3). The purple landraces showed complete resistance to zeocin (landrace P1) or full DNA repair after 1-h rest (landrace P2), whereas inbred lines showed only partial DNA repair (B73) or no repair (Mo17).

A similar result was observed for genes *ZeaRAD51* and *ZeaBRCA1* (Fig. 4), Notably polymorphisms were observed in sequences near and within the FATC domain of the ATM1 kinase gene (Fig. 5), raising the possibility of a functional link between polymorphisms and resistance to zeocin

Alignment and comparison of aminoacid residues in ATM1 within the FATC domain of the ATM1 protein of maize inbred lines (B73 and Mo17), purple Central American landraces (P1 and P2) and *A. thaliana* suggested the existence of two amino acid substitutions: S for V (Ser for Val) and Q for R (Gln for Arg). The FATC domain is believed to mediate the activation of ATM1 under DNA damage (Sun et al. 2018).

Discussion

Our results from comet assay experiments suggested complete resistance to zeocin (landrace P1) or full DNA repair after a 1-h rest (landrace P2), whereas inbred lines showed only partial DNA repair (B73) or no repair (Mo17). High-resolution melting-curve results also suggested the presence of polymorphisms in the genes *ZeaATM1*, *ZeaSOG1, ZeaRAD51* and *ZeaBRCA1* in the Costa Rican purple landraces P1 and P2.

Nonetheless, the values of DNA damage we observed are lower than those previously reported for *A. thaliana* with ionizing radiation or other radiomimetic agent treatment (Menke et al. 2001; Kozak et al. 2009). In our study, the maximum tail DNA values ranged from 2.95 to 4.95%, whereas *A. thaliana* seedlings exposed to bleomycin (0.25–1 μg^{-mL}/1 h) showed 15–30% DNA in the nuclear tail (Menke et al. 2001). Bleomycin is a glycopeptide from the same family as zeocin and with the same mechanism of action (Hu et al. 2018). Differences in root absorption, vascular diffusion and the presence of secondary metabolites may account for these differences in results. Thus, DNA recombination and repair in maize may be efficient with a combination of both chemoprotective measures, such as flavanone 3′-hydroxylase–dependent anthocyanin synthesis (Petroni et al. 2014); improved kinetic efficiency in ATM-mediated repair of DSBs, as observed in radioresistant human breast cancer cells (Bian et al., 2020); and crosstalk between pathways (Verma et al. 2020).

High-resolution melting-curve results suggested that the putative sequences for genes *ATM, SOG1, BRCA1* and *RAD51* (i.e., *ZeaATM1*, *ZeaSOG1*, *ZeaBRCA1* and *ZeaRAD51* in maize) in Costa Rican purple landraces P1 and P2 feature polymorphisms that presumably promote HR-dependent repair of DSBs. No such results were obtained for genes that mediate non-homologous end-joining, such as *KU80* and *LIG4* (data not shown), so in Central American landraces, repair of DSBs caused by zeocin may rely primarily on HR. However, repair of DSBs in rice (caused by bleomycin at 80 μg^{-mL}) relied mostly on KU80-mediated non-homologous

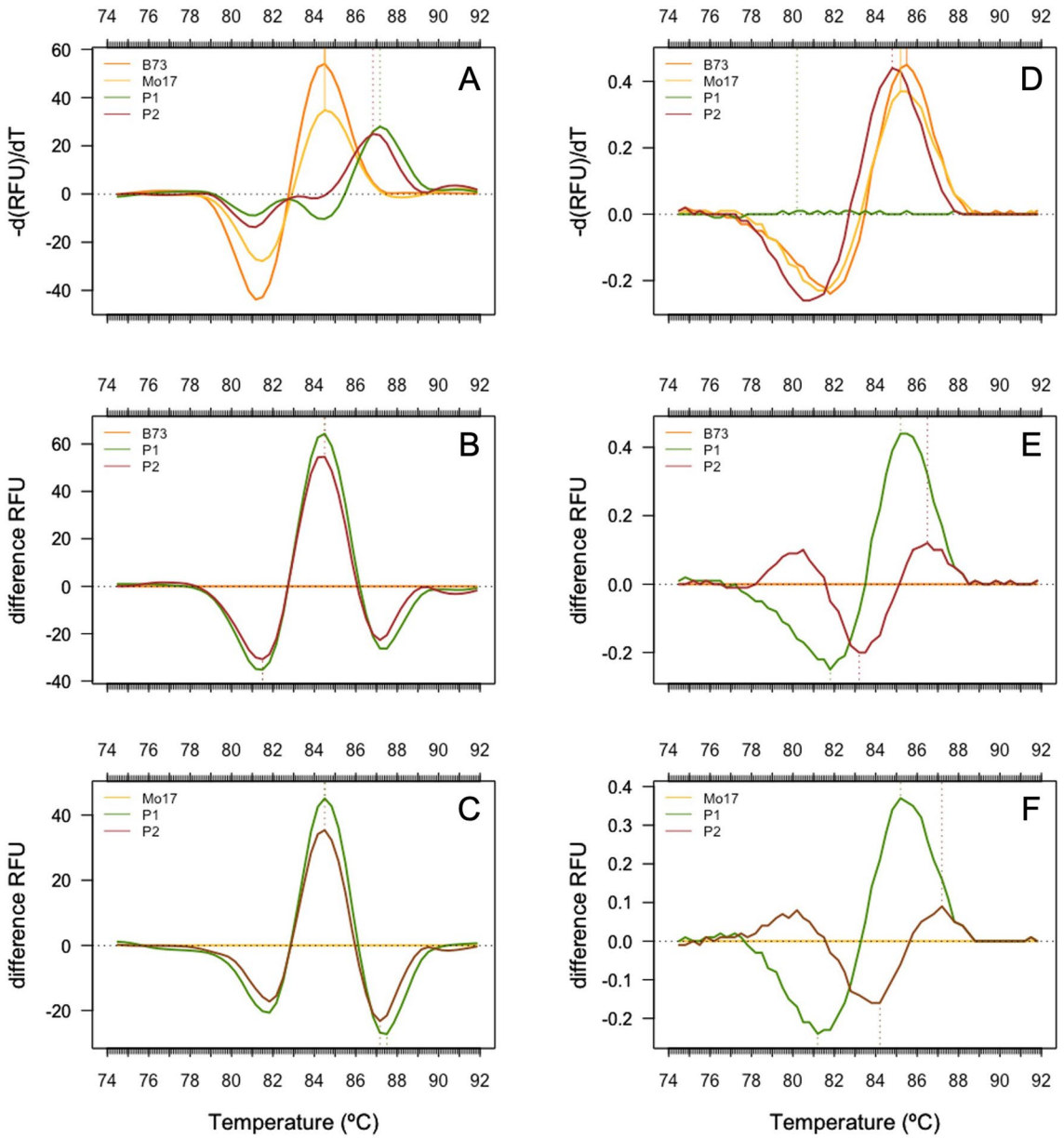

Fig. 3 High-resolution melting curve analysis of putative DNA-repair genes in maize: *ZeaATM1* and *ZeaSOG1*. Melting curves were created as a negative first derivative (− d(RFU)/d(T)) using non-normalized relative fluorescence values (RFUs) for *ATM1* (**A**) and *SOG1* (**D**). Difference curves are shown for inbred lines (B73 or Mo17) against purple landraces (P1 and P2) for *ATM1* (**B** and **C**, respectively) and *SOG1* (E and F, respectively) ($n = 3$, 3 biological samples and 3 technical replicates)

end-joining, and there is evidence of complementation and competition with RAD51-mediated HR. Hence, in eukaryotes, the relationship is overly complex (Xu et al. 2018). A query of putative alleles in the genome of the nested association mapping founder lines from CIMMYT (Mexico) with the MaizeGDB genetic information tool (Portwood et al. 2019) did not return any results for *ZeaATM1* or *ZeaSOG1,* (https://www.maizegdb.org/gene_center/gene/GRMZM2G004593 and https://www.maizegdb.org/gene_center/gene/GRMZM2G027309), so the genomes of P1 and P2 may be evolutionarily distinct and might have developed unique mechanisms to

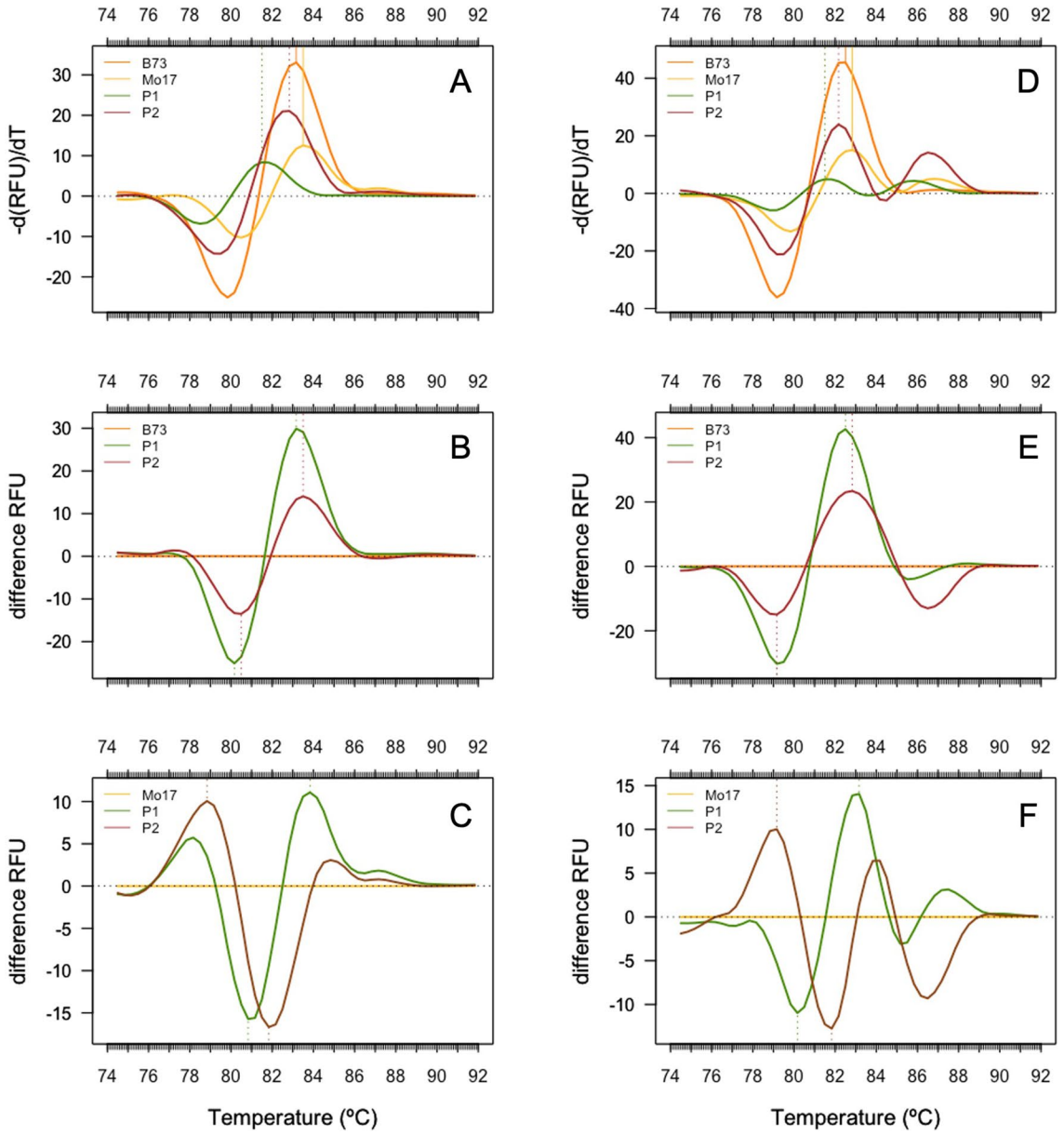

Fig. 4 High-resolution melting curve analysis of putative DNA-repair genes in maize: *ZeaRAD51* and *ZeaBRCA1*. Melting curves were created as a negative first derivative (−d(RFU)/d(T)) using non-normalized relative fluorescence values (RFUs) for *RAD51* (**A**) and *BRCA1* (**D**). Difference curves are shown for inbred lines (B73 or Mo17) against purple landraces (P1 and P2) for *RAD51* (**B** and **C**, respectively) and *BRCA1* (E and F, respectively) ($n = 3$, 3 biological samples and 3 technical replicates)

cope with damage to DNA. For *ZeaATM1*, sequencing results from P1 and P2 indicate the existence of putative non-synonymous polymorphisms right before the MRSIH (MRVKQKLDGYEGGEMRSIH) sequence of the FATC domain of this kinase, in particular Q for T (Gln for Thr) and G for D (Gly for Asp), and two within the FATC domain itself: S for V (Ser for Val) and Q for R (Gln for Arg). Previous results in human HeLa cells by Sun et al. (2005) indicate that this highly conserved domain is required for the successful activation (under DNA

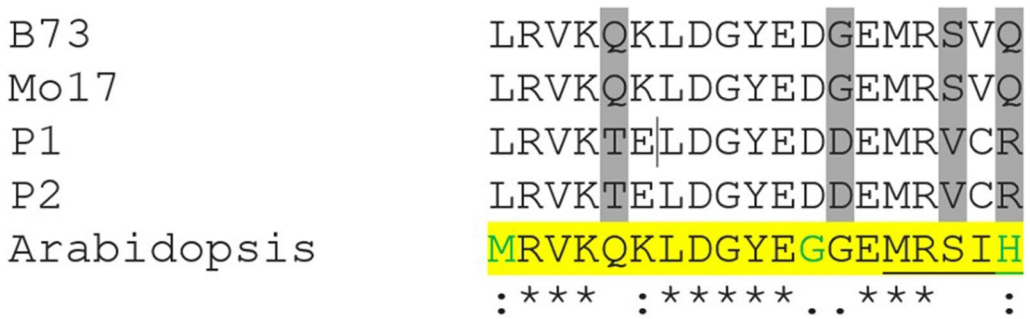

Fig. 5 Alignment of aminoacid sequences adjacent to and within the FATC domain of the ATM1 kinase protein (residues MRSIH, underlined) of maize inbred lines (B73 and Mo17), purple Central American landraces (P1 and P2) and *Arabidopsis thaliana*

damage) of ATM by the histone acetyltransferase Tip60. In fact, failure to interact with Tip60 leads to increased sensitivity to the radiomimetic agent bleomycin and to gamma radiation as well as poor localization to sites of damage labeled by the γ-H2AX marker (Sun et al. 2005).

Work by Pedrosa-Garcia et al. (2021) in the inbred maize line B104 suggested that repair of DSBs on DNA caused by zeocin (75–150 μM) may depend primarily on the activity of the kinase gene ATM and Rad3-related (*ATR*), a gene supposed to mediate repair of single-strand breaks, and not *ATM*. This conclusion is supported by several lines of evidence in the CRISPR-Cas9–derived *ZeaATR and ZeaATM* mutants, especially the so-called *zmatrb* homozygous line, which showed severe root-growth impairment, hyperaccumulation of γH2AX foci, and reduced transcription of *RAD51* and *BRCA1* (Pedrosa-Garcia et al. 2021). Defective transcription of *RAD51* and *BRCA1* was interpreted as suggesting that a downstream response to replicative stress is crucial during DSB repair in maize (Pedrosa-Garcia et al. 2021).

Our experimental results are preliminary, may need to be corroborated by extensive sequencing and by no means rule out that in Central American maize landraces, DNA damage induced by zeocin may be repaired by mechanisms other than homologous recombination, perhaps in an overlapping manner, or by crosstalk between pathways. Nonetheless, our results suggest that some maize genotypes could be more resistant to DNA damage than others, a conclusion supported by a previous phenotypic screening of maize lines exposed to gamma-radiation at 15 Gy: another Costa Rican purple landrace showed better root elongation than B73 (Bolaños-Villegas, 2018).

Conclusions

Our results suggest key adaptive differences in somatic DNA repair efficiency between inbred lines and landraces of maize. Some Central American landraces might be resistant or partially tolerant to DNA damage caused by zeocin. High-resolution melting-curve analysis and sequencing data suggested that the purple landraces P1 and P2 may harbor DNA polymorphisms in genes required for homology-dependent DNA repair such as *ZeaATM1*, which encodes a kinase, and downstream recombination factors such as *ZeaRAD51* and *ZeaBRCA1*. These landraces also showed partial or full resistance to the radiomimetic agent zeocin, contrary to what occurred in other accessions. Nonetheless, confirmation of this result and identification of the actual repair mechanisms requires detailed sequencing and functional characterization. Also, how repair may operate across different tissues, such as the germ line, is still unknown.

Central American landraces may be subject to continuous environmental pressure and agricultural selection by farmers for tolerance to DNA damage, and such traits may be inheritable and possibly subject to transmission into commercial inbred lines. The identification of key loci or quantitative trait loci in these landraces for conventional plant breeding of enhanced DNA repair may be a straightforward way to maintain crop yield during unfavorable conditions. Nonetheless, the government of Costa Rica does not run an official seed bank for preserving and distributing maize landraces. Our results suggest that Central American local maize landraces may constitute a pool of genetic diversity with considerable biological and commercial value. This observation deserves governmental attention as part of a strategy toward ensuring sustainability through climate action, responsible use of terrestrial ecosystems and promotion of food security.

Acknowledgements
We thank Vicerrectoría de Investigación, University of Costa Rica for their financial support.

Author contributions
CVZ performed all experiments, reproduced the stocks, and collected seed; SCP reproduced the B73 and Mo17 stocks; CVZ, MV and PVB designed the experiments; all wrote the manuscript. All authors read and approved the final manuscript.

Authors information
PVB is a Latin American affiliate of the American Society of Plant Biologists.

Funding
This work was supported by grant #B6602 from Vicerrectoría de Investigación of the University of Costa Rica (2016–2019).

Availability of data and materials
Data is available by request; seed stocks are stored at the Centro para Investigaciones en Granos y Semillas (CIGRAS), University of Costa Rica and are available upon request.

Declarations

Ethics approval and consent to participate
This work received approval by the biodiversity commission of the University of Costa Rica.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 25 August 2022 Accepted: 17 October 2022
Published online: 29 October 2022

References

Arford MR, Horn SP. Pollen evidence of the earliest maize agriculture in Costa Rica. J Latin Amer Geo. 2004;3:108–15. https://doi.org/10.1353/lag.2005.0001.

Bedoya CA, Dreisigacker S, Hearne S, Franco J, Mir C, Prasanna BM, Taba S, Charcosset A, Warburton ML. Genetic diversity and population structure of native maize populations in Latin America and the Caribbean. PLoS ONE. 2017;12:e0173488. https://doi.org/10.1371/journal.pone.0173488.

Bian L, Meng Y, Zhang M, Guo Z, Liu F, Zhang W, Ke X, Su YX, Wang M, Yao Y. *ATM* expression is elevated in established radiation-resistant breast cancer cells and improves DNA repair efficiency. Int J Biol Sci. 2020;16:1096–106. https://doi.org/10.7150/ijbs.41246.

Biever JJ, Brinkman D, Gardner G. UV-B inhibition of hypocotyl growth in etiolated *Arabidopsis thaliana* seedlings is a consequence of cell cycle arrest initiated by photodimer accumulation. J Exp Bot. 2014;65(11):2949–61. https://doi.org/10.1093/jxb/eru035.

Bolaños-Villegas P. The study of reproduction of stress tolerance in tropical crops as a means to achieve zero hunger [version 1; not peer reviewed]. F1000Research. 2018;7:1954. https://doi.org/10.7490/f1000research.1116362.1.

Cairns JE, Prasanna BM. Developing and deploying climate-resilient maize varieties in the developing world. Curr Opin Plant Biol. 2018;45:226–30. https://doi.org/10.1016/j.pbi.2018.05.004.

Casati P, Walbot V. Differential accumulation of maysin and rhamnosyl isoorientin in leaves of high-altitude landraces of maize after UV-B exposure. Plant Cell Environ. 2005;28:788–99. https://doi.org/10.1111/j.1365-3040.2005.01329.x.

Chankova SG, Dimova E, Dimitrova M, Bryant P. Induction of DNA double-strand breaks by Zeocin in *Chlamydomonas reinhardtii* and the role of increased DNA double-strand breaks rejoining in the formation of an adaptive response. Radiat Environ Biophys. 2007;46:409–16. https://doi.org/10.1007/s00411-007-0123-2.

Di Rienzo JA, Guzmán AW, Casanoves F. A multiple-comparisons method based on the distribution of the root node distance of a binary tree. J Agric Biol Environ Stat. 2002;7:129–42.

Diffey BL. Sources and measurement of ultraviolet radiation. Methods. 2002;28:4–13.

Dorn A, et al. An Arabidopsis FANCJ helicase homologue is required for DNA crosslink repair and rDNA repeat stability. PLOS Genet. 2019;15(5):e1008174. https://doi.org/10.1371/journal.pgen.1008174.

Eli RM, Daniel LV, Hank WB, Edward SB. Open chromatin reveals the functional maize genome. Proc Natl Acad Sci USA. 2016;113(22):E3177–84. https://doi.org/10.1073/pnas.1525244113.

Horn S, et al. Pre-Columbian maize agriculture in Costa Rica, pollen and other evidence from lake and swamp evidence. In: Staller J, et al., editors. Histories of maize: multidisciplinary approaches to the prehistory, biogeography, domestication, and evolution of maize. San Diego: Elsevier Press; 2006. p. 367–80.

Hu Q, Xie Y, Ge Y, Nie X, Tao J, Zhao Y. Resting T cells are hypersensitive to DNA damage due to defective DNA repair pathway. Cell Death Dis. 2018;9:662. https://doi.org/10.1038/s41419-018-0649-z.

Hufford MB, et al. Comparative population genomics of maize domestication and improvement. Nat Genet. 2012;44:808–11. https://doi.org/10.1038/ng.2309.

Jian Y, Xu C, Guo Z, Wang S, Xu Y, Zou C. Maize (*Zea mays* L.) genome size indicated by 180-bp knob abundance is associated with flowering time. Sci Rep. 2017;7:1–9. https://doi.org/10.1038/s41598-017-06153-8.

Kennett DJ, Thakar HB, Van Derwarker AM, Webster DL, Culleton BJ, Harper TK, Kistler L, Scheffler TE, Hirth K. High-precision chronology for Central American maize diversification from El Gigante rock shelter, Honduras. Proc Natl Acad Sci USA. 2017;114:9026–31. https://doi.org/10.1073/pnas.1705052114.

Ko Y, Kimura S, Maki H, Britt AB, Umeda M. The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. Plant Signal Behav. 2014;9:1–8. https://doi.org/10.4161/psb.28889.

Kozak J, West CE, White C, Da Costa-Nunes JA, Angelis KJ. Rapid repair of DNA double strand breaks in *Arabidopsis thaliana* is dependent on proteins involved in chromosome structure maintenance. DNA Repair (amst). 2009;8:413–9. https://doi.org/10.1016/j.dnarep.2008.11.012.

Kristler L, Thakar HB, Van Derwarker AM, Domic A, Bergström A, George RJ, Harper TK, Allaby RG, Hirth K, Kennett DJ. Archaeological Central American maize genomes. Proc Natl Acad Sci USA. 2020;117(52):33124–9. https://doi.org/10.1073/pnas.2015560117.

Krupa SV, Jäger HJ. Adverse effects of elevated levels of ultraviolet (UV)-B radiation and ozone (O_3) on crop growth and productivity. In: Bazzaz F, Sombroek W, editors. Global climate change and agricultural production. Chichester: FAO and John Wiley & Sons; 1996. p. 141–69.

Kurzbauer MT, Janisiw MP, Paulin LF, Prusén Mota I, Tomanov K, Krsicka O, Von Haeseler A, Schubert V, Schlögelhofer P. ATM controls meiotic DNA double-strand break formation and recombination and affects synaptonemal complex organization in plants. Plant Cell. 2021;33(5):1633–56. https://doi.org/10.1093/plcell/koab045.

Landry LG, Stapleton AE, Lim J, Hoffman P, Hays JB, Walbot V, Last RL. An Arabidopsis photolyase mutant is hypersensitive to ultraviolet-B radiation. Proc Natl Acad Sci U S A. 1997;94(1):328–32. https://doi.org/10.1073/pnas.94.1.328.

Lim G, Chang Y, Huh WK. Phosphoregulation of Rad51/Rad52 by CDK1 functions as a molecular switch for cell cycle–specific activation of homologous recombination. Sci Adv. 2020;6(6):eaay2669. https://doi.org/10.1126/sciadv.aay2669.

Lindfors A, Arola A. On the wavelength-dependent attenuation of UV radiation by clouds. Geophys Res Lett. 2008;35(5):1–5. https://doi.org/10.1029/2007GL032571.

Menke M, Chen IP, Angelis KJ, Schubert I. DNA damage and repair in *Arabidopsis thaliana* as measured by the comet assay after treatment with different classes of genotoxins. Mutat Res Genet Toxicol Environ Mutagen. 2001;493:87–93. https://doi.org/10.1016/S1383-5718(01)00165-6.
Meschichi A, Reeck S, Sicard A, Pontvianne F, Rosa S. The plant-specific DDR factor SOG1 increases chromatin mobility in response to DNA damage. bioRxiv. 2021. https://doi.org/10.1101/2021.11.03.466744.
Møller P, et al. Minimum Information for Reporting on the Comet Assay (MIRCA): recommendations for describing comet assay procedures and results. Nat Protoc. 2020;15:3817–26. https://doi.org/10.1038/s41596-020-0398-1.
Németh P, Tóth Z, Nagy Z. Effect of weather conditions on UV-B radiation reaching the earth's surface. J Photochem Photobiol B: Biol. 1996;32(3):177–81. https://doi.org/10.1016/1011-1344(95)07221-7.
Nisa Mu, Huang Y, Benhamed M, Raynaud C. The plant DNA damage response: signaling pathways leading to growth inhibition and putative role in response to stress conditions. Front Plant Sci. 2019;10:653. https://doi.org/10.3389/fpls.2019.00653.
Panhwar QA, Naher UA, Radziah O, Shamshuddin J, Razi IM. Eliminating aluminum toxicity in an acid sulfate soil for rice cultivation using plant growth promoting bacteria. Molecules. 2015;20(3):3628–46. https://doi.org/10.3390/molecules20033628.
Pedroza-Garcia JA, Eekhout T, Achon I, Nisa MU, Coussens G, Vercauteren I, Van den Daele H, Pauwels L, Van Lijsebettens M, Raynaud C, De Veylder L. Maize ATR safeguards genome stability during kernel development to prevent early endosperm endocycle onset and cell death. Plant Cell. 2021;33(8):2662–84. https://doi.org/10.1093/plcell/koab158.
Petroni K, Pilu R, Tonelli C. Anthocyanins in corn: a wealth of genes for human health. Planta. 2014;240:901–11. https://doi.org/10.1007/s00425-014-2131-1.
Pfeffer CM, Ho BN, Singh ATK. The evolution, functions, and applications of the breast cancer genes BRCA and BRCA. Cancer Genomics Proteomics. 2017;14:293–8.
Portwood JL II, et al. MaizeGDB 2018: the maize multi-genome genetics and genomics database. Nucleic Acids Res. 2019;47:D1146–54. https://doi.org/10.1093/nar/gky1046.
Ray DK, Mueller ND, West PC, Foley JA. Yield trends are insufficient to double global crop production by 2050. PLoS ONE. 2013;8:e66428. https://doi.org/10.1371/journal.pone.0066428.
Ries G, Buchholz G, Frohnmeyer H, Hohn B. UV-damage-mediated induction of homologous recombination in *Arabidopsis* is dependent on photosynthetically active radiation. Proc Natl Acad Sci USA. 2000;97(24):13425–9. https://doi.org/10.1073/pnas.230251897.
Rundell MS, Wagner ED, Plewa MJ. The comet assay: genotoxic damage or nuclear fragmentation? Environ Mol Mutagen. 2003;42:61–7. https://doi.org/10.1002/em.10175.
Sakamoto T, Sakamoto Y, Grob S, Slane D, Yamashita T, Ito N, Oko Y, Sugiyama T, Higaki T, Hasezawa S, Tanaka M, Matsui A, Seki M, Suzuki T, Grossniklaus U, Matsunaga S. Two-step regulation of centromere distribution by condensin II and the nuclear envelope proteins. Nat Plants. 2022;8(8):940–53. https://doi.org/10.1038/s41477-022-01200-3.
Scheben A, Edwards D. Towards a more predictable plant breeding pipeline with CRISPR/Cas-induced allelic series to optimize quantitative and qualitative traits. Curr Opin Plant Biol. 2018;45:218–25. https://doi.org/10.1016/j.pbi.2018.04.013.
Schiwek S, Beule L, Vinas M, Pfordt A, Von Tiedemann A, Karlovsky P. High-Resolution Melting (HRM) curve assay for the identification of eight *Fusarium* species causing ear rot in maize. Pathogens. 2020;9:270. https://doi.org/10.3390/pathogens904027.
Schnable PS, et al. The B73 maize genome: complexity, diversity, and dynamics. Science. 2009;326(5956):1112–5. https://doi.org/10.1126/science.1178534.
Schneider T, Kaul CM, Pressel KG. Possible climate transitions from breakup of stratocumulus decks under greenhouse warming. Nat Geosci. 2019;12:163–7. https://doi.org/10.1038/s41561-019-0310-1.
Schröpfer S, Knoll A, Trapp O, Puchta H. DNA repair and recombination in plants. In: Howell S, editor. Molecular biology. New York: Springer; 2014. p. 51–93.
Sjögren CA, Larsen PB. SUV2, which encodes an ATR-related cell cycle checkpoint and putative plant ATRIP, is required for aluminium-dependent root growth inhibition in Arabidopsis. Plant Cell Environ. 2017;40(9):1849–60. https://doi.org/10.1111/pce.12992.
Sun Y, Jiang X, Chen S, Fernandes N, Price BD. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc Natl Acad Sci USA. 2005;102(37):13182–7. https://doi.org/10.1073/pnas.050421110.
Sun S, et al. Extensive intraspecific gene order and gene structural variations between Mo17 and other maize genomes. Nat Genet. 2018;50:1289–95. https://doi.org/10.1038/s41588-018-0182-0.
Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673–80. https://doi.org/10.1093/nar/22.22.4673.
Verma P, Tandon R, Yadav G, Gaur V. Structural aspects of DNA repair and recombination in crop improvement. Front Genet. 2020;11:574549. https://doi.org/10.3389/fgene.2020.574549.
Wang Y, Wang Q, Huang H, Huang W, Chen Y, McGarvey PB, Wu CH, Arighi CN, UniProt Consortium. A crowdsourcing open platform for literature curation in UniProt. PLOS Biol. 2021;19(12):e3001464. https://doi.org/10.1371/journal.pbio.3001464.
Wheeler T, Braun V. Climate change impacts on global food security. Science. 2013;341(6145):508–13. https://doi.org/10.1126/science.1239402.
Wijewardana C, Henry WB, Gao W, Reddy KR. Interactive effects on CO_2, drought, and ultraviolet-B radiation on maize growth and development. J Photochem Photobiol b, Biol. 2016;160:198–209. https://doi.org/10.1016/j.jphotobiol.2016.04.004.
Xu Z, Zhang J, Xu M, Ji W, Yu M, Tao Y, Gong Z, Gu M, Yu H. Rice RAD51 paralogs play essential roles in somatic homologous recombination for DNA repair. Plant J. 2018;95(2):282–95. https://doi.org/10.1111/tpj.13949.

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