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# DNA DAMAGE RESPONSE DURING EARLY GERMINATION IN RELATION TO SEED AGING AND RADIO-SENSITIVITY IN BARLEY

Accumulation of DNA damage is a hallmark of aged seeds; therefore, the successful repair during imbibition is an important prerequisite for seed germination and further seedling development. In this work we investigated the effects of aging and ionizing radiation on the germination potential of barley seeds and their ability to activate cellular DNA damage repair response systems. Aged and unaged seeds were subjected to 100 Gy X-ray irradiation for 5 hours after imbibition and the accumulation of DNA lesions and the activation of DNA repair mechanisms were observed and recorded; 2, 6 and 24 hours afterwards. The levels of oxidative DNA damage were measured at the whole genome level by alkaline gel-electrophoresis and in specific nuclear and extra-nuclear genes by Long-range PCR. Transcriptional profiles of barley gene homologues involved in various DNA repair pathways were assessed by semi-quantitative and quantitative RT-PCR. Actin, tubulin and 18S rRNA genes were tested as reference controls for the time periods analysed. Accelerated aging increased the radio-sensitivity of imbibed barley seeds in terms of delayed germination and reduced growth. The results obtained with naturally aged seeds showed the presence of formamidopyrimidine-DNA glycosylase (FPG)-sensitive sites in barley nuclear, chloroplast and mitochondrial genomes early after imbibition in both irradiated and control seeds and lesions were still detectable at the 24h time-point. In response to ionizing radiation RAD51 and PARP2 were found to be specifically modulated during the early imbibition periods. The obtained results reveal molecular mechanisms which might underlie the tolerance of aged and unaged barley seeds to ionizing radiation. Knowledge on the processes responsible for the fate of DNA damage during seed germination has the potential to influence the efficiency of mutation induction protocols utilized for breeding purposes based on the irradiation of dry seeds.

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