

'% DNA in tail' values from about 2–4. The intercellular (intraindividual) heterogeneity in DNA migration thereafter increased to reach an average deviation of 15 as a result of a fraction of cells with (still) excessive DNA migration besides cells with repaired DNA damage (Fig. 1). The root-to-root (interindividual) variability also increased after 6 h of imbibition, which might be explained by differences in vigour of the seed embryos.

During imbibition of the seeds, BrdU labelling was applied. When analysing DNA with SCGE, the position of the repair sites could be visualized in the comet migration patterns. However, BrdU incorporation is non-specific and appears during replication and repair synthesis (unscheduled DNA synthesis). Depending on the species and the vigour of the embryo, the first cycle of replicative DNA synthesis may occur 4–24 h after imbibing in water (Elder and Osborne, 1993). For field bean, an onset of DNA synthesis was reported after 25 h at 25 °C (Jacob and Bovey, 1976) or 36–40 h at 22 °C (Angelis et al., 1986). Kuglík et al. (1988) suggested to make use of this pre-replicative period in radicle cells of germinating seeds to distinguish unscheduled and scheduled (replicative) DNA synthesis. Intensity and location of BrdU incorporation in the comet patterns changed with the time of imbibition. It was interpreted as a result of altering DNA repair and replication activities. During the first 15 h, BrdU-labelled DNA regions were observed most probably solely due to DNA repair and were localized all over the entire comet (Fig. 2a,b). Between 15 and 27 h post-imbibition time, DNA migration levelled off (Fig. 1). Possibly, single-strand breaks caused by DNA replication enhanced DNA mobility and therefore 'covered' the effect of the DNA repair process (Olive and Banáth, 1993; Šalagovic et al., 1997). Substantially more BrdU was incorporated, which was most probably due to replication (Fig. 2c,d). From 27 to 33 h post-imbibition time, DNA migration was again lower, repair synthesis was considerably decreased and in most cells BrdU was mainly incorporated in the comet 'head' region (Fig. 2e,f). Klaude et al. (1996) found, after BrdU-pulse labelling of proliferating Chinese hamster ovary cells, replicated (labelled) regions only in the heads of the comets. They explained it by comets consisting of DNA loops fixed to some structure in the nucleus, where also the DNA synthesis by the immobile replication machinery takes place.

As a conclusion it may be stressed that the alkaline single-cell gel electrophoresis assay is a suitable tool to determine DNA integrity in individual cells of germinating seeds. Applying BrdU labelling allowed visualization of repair patches localized over the entire comet migration pattern of the cells.

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