Results and Discussion

DNA damage in seeds was studied before (e.g. Rhoderick and Osborne, 1993). The alkaline SCGE assay provides an easy way to assess DNA migration in individual nuclei. The more DNA is damaged, the higher DNA mobility in the electric field. In Fig. 1 the 'median % DNA in the tail' values for five different *Vicia faba* radicles per time point of imbibition were plotted. In dry seeds ca 90% DNA was found in the tail of the comet migration pattern, which means high damage (Fig. 1). Spontaneous base damage reported by Dandoy et al. (1987) might be an important cause of DNA degradation when storing dry maize seeds. These altered bases were removed during the first hours of rehydration, leaving (alkali-labile) apurinic/apyrimidinic (AP) sites. Under alkaline conditions, alkali-labile sites are converted into breaks, which enhance DNA mobility during electrophoresis. Besides that, ca 6 strand breaks per 10^8 dalton/year occur spontaneously in seed DNA (McLennan, 1988). *Vicia faba* contains 29 pg DNA (Bryant, 1976); this means more or less 1.05×10^6 breaks per year. For comparison, 10 Gray of X-rays induce approximately 2 single-strand breaks (ssb) per 10^9 dalton DNA (Howland et al., 1975; Ahnström and Erixon, 1981), or for *Vicia faba* approximately 3500 ssb per Gy and cell. Two-year storage of the seeds therefore induced a DNA damage more or less equal to an irradiation dose of 600 Gy.

The germination capacity of the seeds was still 95%. This was because of DNA repair at the onset of seed germination (Osborne et al., 1980; McLennan, 1988; Velemínský and Angelis, 1990). DNA repair can be followed with the alkaline comet assay. The average median of 80–90% DNA in the 'tail' of the comets from radicle cells imbibed during the first 6 h dropped very quickly to 50% at 9 h of imbibition (Fig. 1). DNA mobility was slowly decreasing to more or less 35% after 15 h, levelled off until 27 h, and dropped again to 20% after 33 h of imbibition. Not all cells were repaired with equal speed as was clear from the range data. Cells of dry or 3 to 6 h imbibed radicles had a homogeneously high DNA migration, with an average deviation of

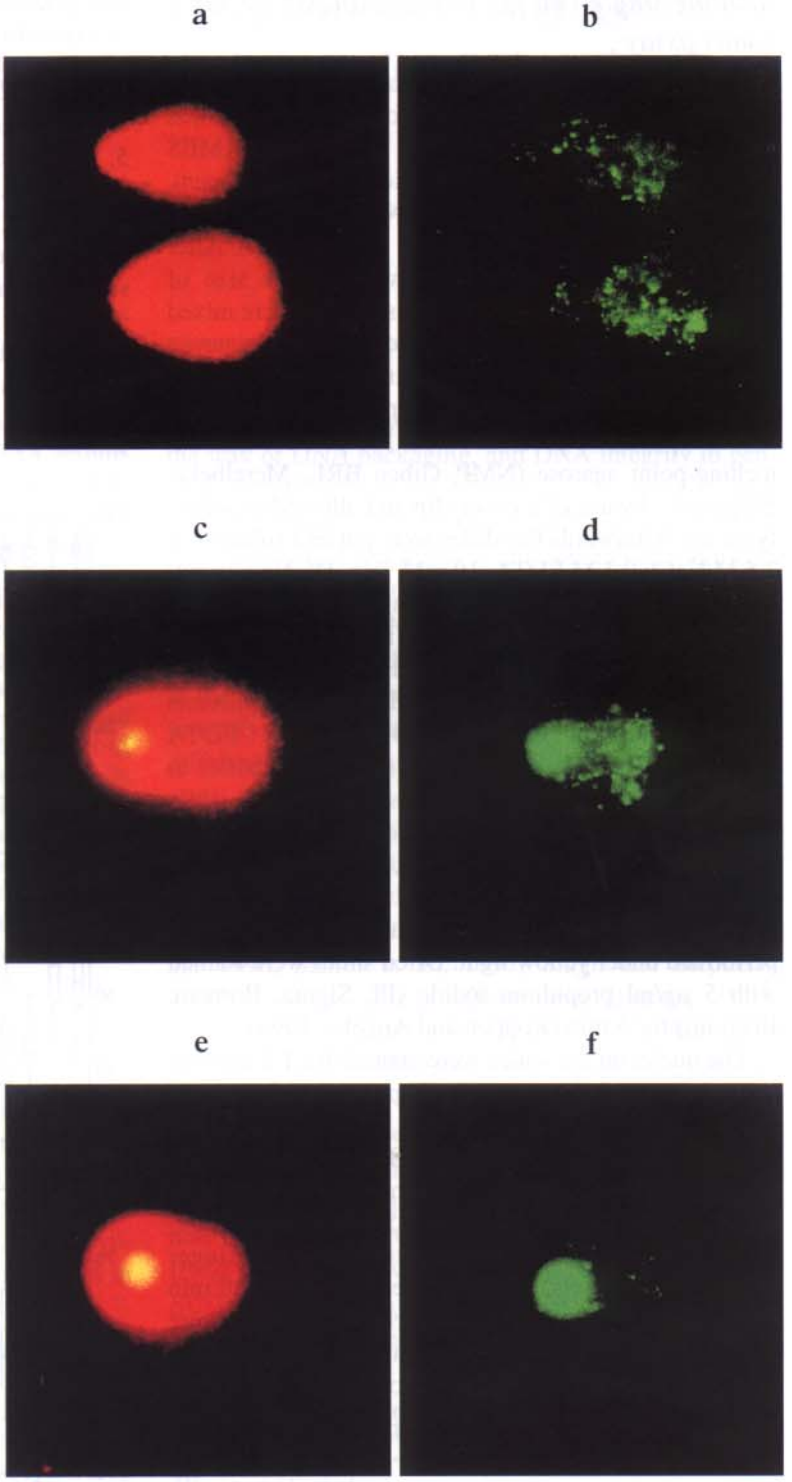

Fig. 2. Photos of comets containing BrdU-labelled regions (6 h BrdU incorporation prior seed collection). BrdU was visualized by staining with FITC-conjugated antibodies. Viewing was done with a Zeiss epifluorescence microscope using a PI filter set 15 (left column) for visualization of the whole DNA, or a FITC filter set 10 (right column) for visualization of the labelled DNA regions only. (a,b) Labelling of comet 'head' and 'tail' during the first 15 h of imbibition. (c,d) Intensive incorporation of BrdU in 'head' and 'tail' 15 to 27 h after onset of rehydration. (e,f) Mainly labelling of 'head' at 30 h post imbibition. The photos were taken with an ASA 400 Fuji colour film at an original magnification of 200x.