Development of an automated scoring system for plant comet assay

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Background

In plants, an increasing interest for the Comet Assay was shown in the last decade and this versatile technique appears to be promising to detect the genotoxic effect of pollutants and to monitor the environment. However, its use in plant studies was rather limited compared to animal studies because of (i) the **difficulty to isolate intact nuclei** compared to animal systems, (ii) the **low throughput of current nucleus extraction**, and (iii) the **lack of a high throughput comet assay scoring method.**

Why automated scoring?

Increasing comet assay throughput requires innovative automated scoring tools which (i) strongly increases overall capacity, (ii) save time, (iii) lower operator bias and (iv) improve robustness of statistical analysis.

Objectives and issues to solve

The **French-Norwegian project ComPack** (2014-2017) aims to deal with these issues. We recently identified the key steps of the comet assay on plant models and proposed an optimized protocol to increase its reliability (Pourrut et al.; 2015). Our next main objectives are:

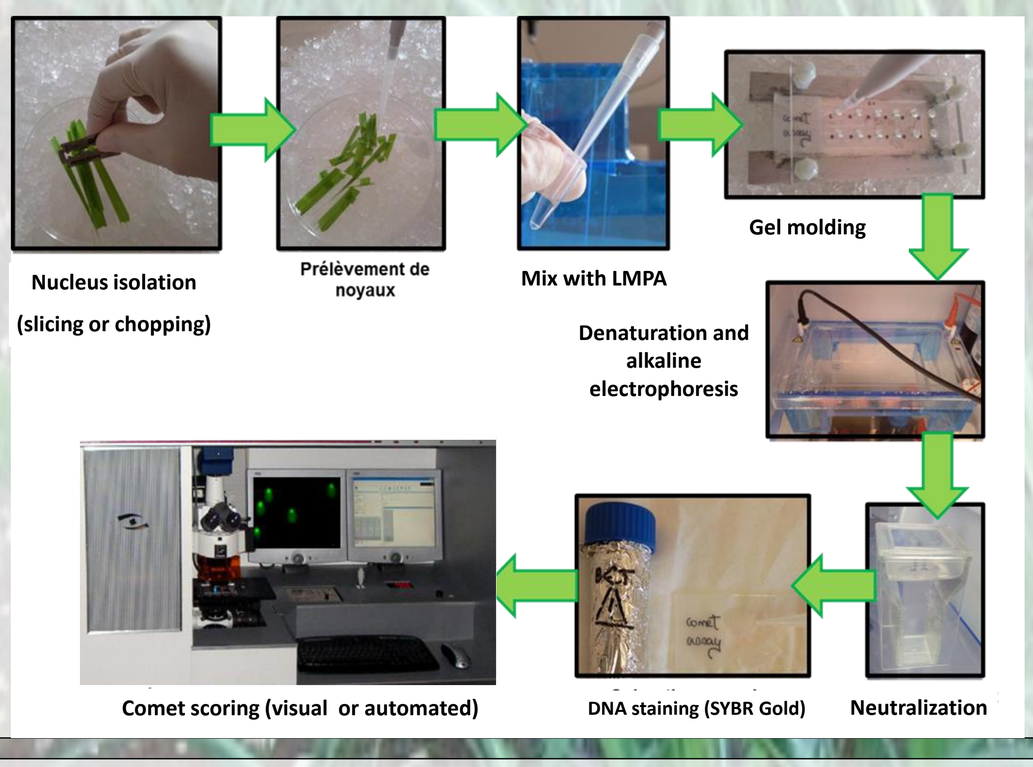
- **the development a new nucleus extraction technique compatible with the high-throughput comet assay scoring methods;**
- **the automation of the scoring method based on the automated scoring system Pathfinder™, developed by IMSTAR.**

Major issues are:

- **increase the nuclei density** is of importance to increase scoring reliability;
- optimization of the protocol to **increase background quality**;
- **specific adaptation of the automated scoring system Pathfinder™** is crucial as it was initially set up for human/animal cells.

Methodology

Material: White clover (*Trifolium repens*) was grown in a greenhouse with controlled conditions for 8 weeks. Soils were watered with a pH 7 water.



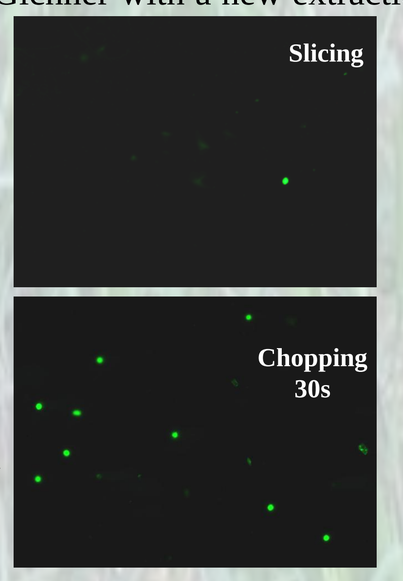
Extraction of nuclei

In plants, the nuclei must be extracted by mechanical procedures. Here we compared the common slicing method developed by Gichner with a new extraction method of the nuclei, by chopping.

Nucleus extraction	Number of nuclei per slide	DNA damage (% DNA in the tail)
Slicing	29.3 ± 12.4 [a]	4.2 ± 0.6 [a]
Chopping 15 s	93.4 ± 11.8 [b]	4.4 ± 0.8 [a]
Chopping 30 s	174.4 ± 14.3 [c]	5.3 ± 0.7 [a]
Chopping 45 s	243.6 ± 31.2 [d]	16.4 ± 2.4 [b]
Chopping 60 s	363.3 ± 35.3 [e]	42.3 ± 5.5 [c]

Table 1: Influence of nucleus isolation methods on nucleus extraction yield and DNA damages of nuclei extracted from control *T. repens* leaves.

Each value represents mean and associated standard error. The letters, a to d, refer to significant differences (Tukey HSD test, $P \leq 0.05$, $n = 6$) between isolation methods

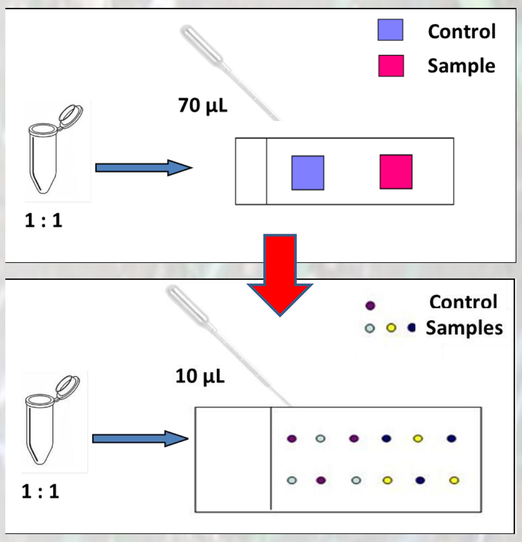


→ **Chopping is clearly a more efficient technique and allows scoring more nuclei. This increases both comet assay reliability and sensitivity**

→ **This opens the possibility of reducing the volume of gels, and thus of increasing comet assay throughput by using the 12-gel slide protocol**

Medium throughput comet assay

To increase throughput, the twelve-gel slide format optimised for comet assay developed by Shaposhnikov *et al.* (2010) was used.



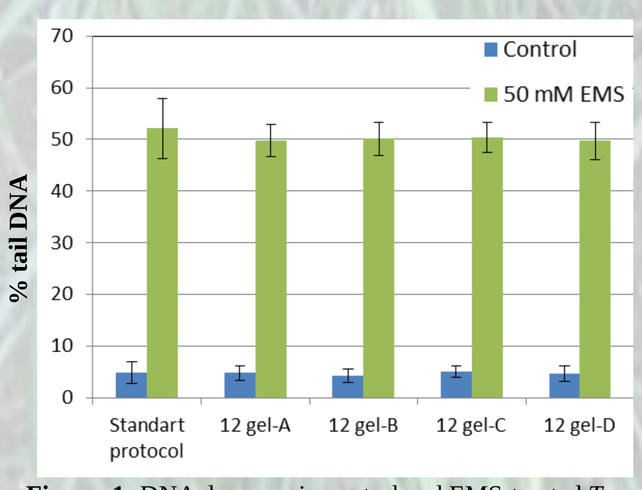


Figure 1: DNA damages in control and EMS-treated *T. repens* plants observed by standard or medium throughput comet assay (gels A to D)

→ **Is as sensitive as standard protocol**

→ **Reduces intra- and inter-experimentation variations**

→ **Is 3 times faster (no coverslip, 1 slide vs 12 in the electrophoresis tank…)**

Pathfinder™ automated scoring system

The existing scoring system devised for human/animal cells needs specific adaptation for use with plant material

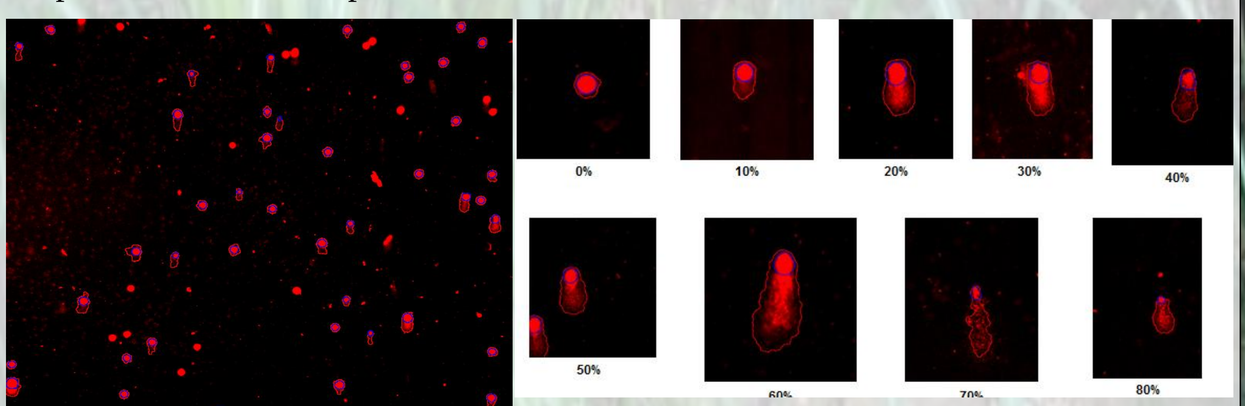


Figure 2 and 3: Analysis of DNA damages observed in control and EMS-treated *T. repens* plants using Pathfinder™ automated scoring system

→ **The Pathfinder™ automated scoring system is clearly compatible with the medium throughput comet assay protocol we developed**

→ **It strongly reduces scoring time (4h vs 10 days)**

→ **However, gel background and image analysis must be improved**

Conclusion

→ **The new nucleus extraction method and the 12-gel protocol increase comet assay reliability, sensitivity and are time-saving**

→ **Our promising preliminary results obtain with the Pathfinder™ automated scoring system open up the perspective of an automated high-throughput scoring of plant nuclei**

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