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The Behavior of a T-DNA Insertion Site from the Genome of Thale Cress (*Arabidopsis thaliana*) under the Putative Mutagenic Effect of Photo-Oxidative Stress

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SUMMARY

The approval of Genetically Modified Plants (GMPs) relies on the assumption that the inserts remain stable in the plant genome. Still, it has been shown that the integration of foreign DNA segments may coincide with minor or major target site re-arrangements and the occurrence of filler DNA segments. Little is known about the effect of conventional agricultural breeding or the influence of environmental conditions or physiological stresses on the integrity and stability of the insert, or the role of the insertion location. Our aim was to study putative sequence changes of a T-DNA insertion site in the genome of thale cress (*Arabidopsis thaliana*) under high light (photo-oxidative) stress, known to induce the formation of genotoxic Reactive Oxygen Species (ROS). One genomic target site was investigated in wild-type (wt) plants (C24 and Col-0 ecotypes) in order to define if and what kind of rearrangements is induced (Windels *et al.*, 2003; De Buck *et al.*, 2004). Plants (C24 and Col-0 ecotype) were grown under regular condition. The control set was brought to maturity in the same conditions, while on the test set the high light treatment was applied just before flowering: 1 day at 300 μmole/m^2/s, followed by 7 days at 600 μmole/m^2/s. The stress applied was visually observed due to the formation of purple colored leaves. The treated plants were retransferred to normal condition. The seeds of all the plants were harvested and the progeny grown in normal conditions. A total of 100 plants/treatment/ecotype were individually analyzed. For the analysis of the target site, a high-throughput method -Single Strand Conformational Polymorphism (SSCP) analysis- was tested and optimized to detect even the minor changes (Single Nucleotide Polymorphisms -SNPs) that could occur. The analysis is based on PCR amplification of the DNA fragment of interest using site-specific dual-fluorescent labeled primers. Fragments are analyzed by means of capillary electrophoresis and fluorescence detection. If changes in the conformational pattern are detected, the DNA fragment is cloned and sequenced to determine the exact nature of the mutation. The results indicate that in wild-type plants, both those grown in standard conditions and those exposed to high light stress, the sequence considered does not undergo any re-arrangement.

REFERENCES

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