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Review

Assessing environmental impacts of genetically modified plants on non-target organisms: The relevance of *in planta* studies

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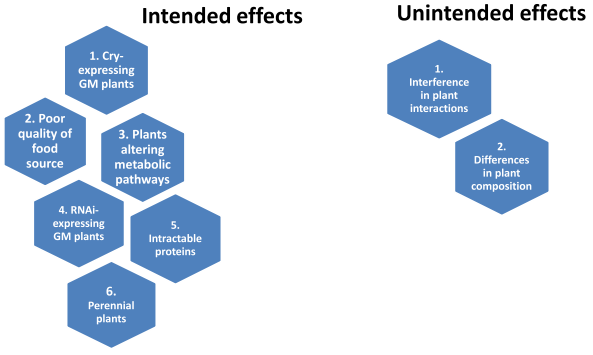
HIGHLIGHTS

- GM plants and their products are possible stressors for non-target organisms.
- In the EU it is compulsory to use GM plants in the experiments for risk assessment.
- Existing literature supports the use of such studies in environmental risk assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

In legal frameworks worldwide, genetically modified plants (GMPs) are subjected to pre-market environmental risk assessment (ERA) with the aim of identifying potential effects on the environment. In the European Union, the EFSA Guidance Document introduces the rationale that GMPs, as well as their newly produced metabolites, represent the potential stressor to be evaluated during ERA. As a consequence, during several phases of ERA for cultivation purposes, it is considered necessary to use whole plants or plant parts in experimental protocols. The importance of *in planta* studies as a strategy to address impacts of GMPs on non-target organisms is demonstrated, to evaluate both effects due to the intended modification in plant phenotype (e.g. expression of Cry proteins) and effects due to unintended modifications in plant phenotype resulting from the transformation process (e.g. due to somaclonal variations or pleiotropic effects). *In planta* tests are also necessary for GMPs in which newly expressed metabolites cannot easily be studied in vitro. This paper reviews the scientific literature supporting the choice of *in planta* studies as a fundamental tool in ERA of GMPs in cultivation dossiers; the

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Ecosystem services
Insect-plant interactions
Non-target organisms
Risk management

evidence indicates they can realistically mimic the ecological relationships occurring in their receiving environments and provide important insights into the biology and sustainable management of GMPs.

Contents

1. Introduction

The evaluation of possible environmental impacts of genetically modified plants (GMPs) and derived food/feed products is an important part of the authorization process, under which such biotechnological innovations are regulated for cultivation by authorities worldwide (e.g. the European Food Safety Authority (EFSA), US EPA, Health Canada, FSANZ). Among other areas of environmental concern (e.g. persistence and invasiveness, horizontal gene transfer, interactions with biogeochemical cycles, etc.), studies on non-target organisms (NTOs) (Arpaia, 2010) are conducted on a regular basis by developers of commercial GM crops in order to assess potential effects of GMPs on biodiversity in and around cultivated areas.

In agro-ecosystems, hundreds of species are sustained in food webs above and below ground, based on cultivated plants as the main primary producers (Mészáros et al., 1984a, 1984b), although most of these species are not economically relevant crop pests, or keystone organisms in supporting Ecosystem Services (Mace et al., 2012). Numerous species at higher trophic levels can come into contact with plants and their metabolites either directly or indirectly, e.g. through feeding on herbivorous hosts/prey (Andow et al., 2006). The concerns arising from the exposure of NTOs to GMPs is that intentional or unintentional changes in expression of some GMP metabolites or changes in plant composition and structure (e.g. lignin, cuticle, hairiness, etc.), may affect ecological interactions and thus ultimately harm sensitive NTOs when sufficiently exposed.

In some areas where GMPs were first cultivated (e.g. USA, Argentina), regulatory bodies adopted a testing system largely based on the eco-toxicological approach, traditionally used for testing noxious chemicals (e.g. pesticides). According to this 'tiered' system, testing starts with small-scale and short-term laboratory tests conducted on surrogate species in which the potentially noxious chemical is mixed with artificial diet in doses much larger than the expected environmental concentration. Only if negative effects on the studied organisms appear at this stage, is scaling up of the experimental setup required to greenhouse semi-field or field experiments using growing plants (Romeis et al., 2008).

In the European Union (EU), the assessment of effects on NTOs in Environmental Risk Assessment (ERA) is discussed in EFSA (2010a, 2010b). These two key Guidance Documents (GDs), adopted by all EU Member States (MS), present guidelines for applicants wishing to introduce GMPs in the European Union for cultivation purposes. The two documents refer to different existing ERA approaches, but also introduce a series of initiatives aimed at further rooting the ERA in sound ecology. These GDs include the requirement to consider the whole GM plant, in addition to the introduced traits (e.g. *Bt* proteins), as the possible environmental stressor. The rationale is that processes involved in genetic modification, as with most other plant breeding techniques, not only introduce intended novel traits but can also cause additional unintended changes to the plant's phenotype which affect its interactions with the receiving environment.

Therefore EFSA (2010a, 2010b) requires the generation of experimental data using viable GM plants or plant parts in the ERA process for cultivation purposes. This provides the basis for a comparative analysis, in which impacts of the GMP are assessed relative to an appropriate (usually genetically similar) non-GM plant comparator and differences are assessed for their potential hazards and risks. *In planta* tests study ecologically representative organisms (e.g. insects, microorganisms) by exposing them to the test plant(s); exposure can be to plant parts or organs (e.g. leaves, stems, seeds, pollen, flowers) or to whole plants in glasshouse, semi-field or field experiments. For NTO risk assessment in cultivation dossiers, *in planta* generated data are deemed necessary in two situations:

1. To evaluate effects on NTOs *due to the intended modification* in plant phenotype (e.g. expression of *Bt* Cry proteins active against certain insect pests);
2. To evaluate possible effects on NTOs *due to unintended modifications* in plant phenotype (e.g. pleiotropic effects, interference with other metabolic pathways, etc.). EFSA (2010b) considers such unintended

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