Unintended phenotypic effects of single gene insertions in potatoes – assessing developmental dynamics and leaf morphology

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Zusammenfassung

In diesem Artikel werden eine Reihe unbeabsichtigter phänotypischer Effekte einer genetischen Modifikation bei der Kartoffelsorte Bintje vorgestellt. In Pflanzen dieser Sorte wurden zwei verschiedene Gen-Konstrukte eingeführt: Ein Viscotoxin Gen aus der Mistel und ein Aminolävulinat Gen aus der Bäckerhefe. Die gentechnisch veränderten (GV) Pflanzen und entsprechende Kontrollen wurden in zwei unterschiedlichen Wuchsbedingungen untersucht. Die GV Pflanzen zeigten Unterschiede in Entwicklungsdynamik und Alterungsprozess; Abweichungen in der Pflanzengestalt, in der Form der Knollen und der Blätter konnten beobachtet werden. Einige Unterschiede in der Blattform zeigten Interaktionen zwischen Pflanze und Umgebung und weisen somit auf eine veränderte Anpassungsplastizität hin.

Die begleitende Untersuchung von phänotypischen Unterschieden zwischen den drei kommerziellen Sorten Bintje, Appell und Naturella ergab, dass das Set der verwendeten morphologischen Charaktere auch geeignet ist, Sortenunterschiede festzustellen. Die Ergebnisse zeigen, dass phänotypische Unterschiede zwischen Pflanzen derselben Sorte mit oder ohne Fremdgen ebenso ausgeprägt sind, wie diejenige zwischen Pflanzen mit unterschiedlichen genetischen Hintergründen.

Die vorliegende Studie steht im Kontrast zu Analysen, in denen das Profil von Eiweissen (Proteom) oder Stoffwechselprodukten (Metabolom) untersucht wurde und in welchen die Unterschiede zwischen den GV Varianten und den Kontrollpflanzen kleiner waren, als diejenigen zwischen Varietäten oder Sorten.

Summary

Unintended phenotypic effects of insertion of the v*iscotoxin gene* from mistletoe and the *aminolevulinate synthase gene* from yeast into the potato (*solanum tuberosum*), cultivar Bintje have been assessed under two different growth conditions. The genetically modified plants exhibit deviations in developmental dynamics and plant senescence. Changes in plant and tuber shape, leaf metamorphosis and leaf architecture are reported. A few traits of leaf architecture reveal plant-environment interactions, and thus hint at a divergent plasticity of the transgenic variants compared to the non-modified controls.

Assessment of phenotypic differences between the cultivars Bintje, Appell and Naturella indicates that the set of morphological characters used in this study is suited to discriminate phenotypes of commercial cultivars. The results show that unintended phenotypic changes due to gene insertion are as pronounced as those due to the different genetic backgrounds.

The present study contrasts analyses of profiling studies of the proteome and metabolome of potatoes, which show that compositional differences between

genetically modified and non-modified variants are smaller than those between varieties and landraces.

Introduction

Risk assessment analysis of genetically modified (GM) crop plants includes food safety, ecological impacts and unintended effects on phenotype and substance composition (*Cellini et al.* 2004; *Haslberger* 2003; *Kuiper et al.* 2001). With the advent of profiling techniques, changes in the spectrum and abundance of transcripts (*Holtorf et al.* 2004), proteins (*Lehesranta et al.* 2005) and metabolites (*Charlton et al.* 2004; *Griffin* 2003; *Le Gall et al.* 2003; *Tretheway* 2004) have been documented. Although such alterations do not suggest a risk *per se*, they are indicative for an integral reaction of the plants upon random insertion of foreign genes and justify paying closer attention to possible adverse effects.

The same holds true for unintended phenotypic effects of genetic modifications, since the phenotype is the manifestation of the plant's developmental history. However, such changes are not as thoroughly investigated as differences in RNA, protein or metabolite composition. Two reasons account for this deficit. First, dependent on the origin of the plant material, planting time, and subtle changes in growth conditions, phenotypes exhibit a pronounced plasticity. Second, during propagation GM crop plants are selected for normal phenotypes over several generations. As a consequence, reports on unintended phenotypic effects are considered to be the exception rather than the rule. But they have repeatedly been documented: dwarfism in tomatoes with constitutive expression of a fruit synthase gene (*Fray et al.* 1995); enhanced cross pollination in *Arabidopsis* with a herbicide resistance gene (*Bergelson et al.* 1998) and reduced seed production (*Purrington and Bergelson* 1999), or multiple effects in soybeans harbouring a herbicide resistance gene (*Gertz et al.* 1999).

In this paper we investigate unintended phenotypic effects in GM potatoes, *Solanum tuberosum*, cv. Bintje, with two gene constructs for putative resistance to late blight (*Phytophtora infestans*), harbouring the *viscotoxin I gene* (Visco) from mistletoe and the *aminolevulinate synthase gene* (Ala) from yeast, respectively (*Malnoë et al.* unpublished results). The study includes the assessment of the developmental dynamics, the plant morphology, the sequence of dried leaves and the architecture of individual leaves (*Bockemühl* 1980; *Holdrege* 1996). Leaf sequences following the order along the shoot axis provide a valuable tool to assess species and plant specific characteristics of leaf formation. They give insight into the formative capacity of the plant and are a sensitive agency for monitoring