Expression of a chimeric stilbene synthase gene in transgenic wheat lines

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Abstract
A chimeric stilbene synthase (sts)-gene was transferred into wheat. Stilbene synthases play a role in the defence against fungal diseases in some plant species (e.g. groundnut or grapevine) by producing stilbene-type phytoalexins like resveratrol. Resveratrol is also claimed to have positive effects to human health. Embryogenic scutellar calli derived from immature embryos of the two commercial German spring wheat cultivars ‘Combi’ and ‘Hanno’ were used as target tissue for co-transformation by microprojectile delivery. The selectable marker/reporter gene constructs contained the bar-gene either driven by the ubiquitin-promoter from maize (pAHC 25, also containing the uidA-gene driven by the ubiquitin-promoter), or by the actin-promoter (pDM 302) from rice. The co-transferred plasmid pStil 2 consisted of a grapevine sts-coding region driven by the ubiquitin promoter. Eight transgenic ‘Combi’ and one ‘Hanno’ T₀-plant were obtained and, except one ‘Combi’ T₀-plant, found to be co-transformants due to the integration of both the sts-gene and the selectable marker or reporter genes. Expression of the sts-gene was proven by RT-PCR, and, for the first time, by detection of the stilbene synthase product resveratrol by HPLC and mass spectrometry. The sts-gene was expressed in four of the seven transgenic ‘Combi’ T₀-plants. Two of the respective T₁-progenies segregated in a Mendelian manner were still expressing the gene. Investigations into methylation of the sts-gene showed that in three non-expressing progenies inactivation was paralleled by methylation.

Introduction
Improvement of disease resistance is one of the aims of plant gene technology. Amongst others, the transfer of stilbene synthase (sts) genes has been studied. Stilbene synthases produce stilbene-type phytoalexins like resveratrol, a substance with fungicidal potential (Langcake and Pryce, 1976). Sts-genes from groundnut (Hain et al., 1990) and grapevine (Hain et al., 1993; Stark-Lorenzen et al., 1997, Fischer et al., 1997) have already been transferred to other plants: tolerance against fungal infections was improved in tobacco (Hain et al., 1993; Fischer et al., 1997), in rice (Stark-Lorenzen et al., 1997), and in barley (Leckband and Lörz, 1998). The sts-gene has also been transferred to wheat, but neither formation of resveratrol nor enhanced fungal resistance was reported (Leckband and Lörz, 1998). In tobacco and petunia, overexpression of sts-genes led to a substrate competition between stilbene and chalkon synthases causing male sterility (Fischer et al., 1997).

In addition, resveratrol may be responsible (for review, see Soleas et al., 1997) for some beneficial effects of red wine on human health, for example inhibition of platelet aggregation (Bertelli et al., 1995), vasorelaxing activity (Chen and Pace-Asciak, 1996), or cancer chemopreventive activity (Jang et al., 1997). Recently, it has been reported that some of these effects could be explained by the stimulation of the estrogen receptor by resveratrol, which acts as a structural analogue of estrogen (Gehm et al., 1997).

Only few years ago, the first successful genetic transformation of wheat was reported. Using Agrobacterium, no stable transformants could be obtained...