UNDECLARED GENETICALLY ENGINEERED ORANGE PETUNIAS HARBOUR A SPECIAL VARIANT OF THE MAIZE DIHYDROFLAVONOL 4-REDUCTASE

Daria Nitarska, Benjamin Walliser, Christian Haselmair-Gosch, Silvija Miosic, Barbara Roth, Karl Stich and Heidi Halbwirth*

[1]Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria

Petunia hybrida belongs to the most commercially important ornamental plants. Despite petunia varieties with various flower colourations being available on the market, orange colouration was missing for a very long time. This is due to the presence of a substrate specific dihydroflavonol 4-reductase (DFR) in petunia, which is not able to convert dihydrokaempferol (DHK) to orange-red pelargonidin type pigments [1]. The first petunia with orange flowers was obtained by a scientific transgenic approach, which introduced the maize *DFR* gene (A_1) in the 1980s [2]. A few years ago orange petunia started to appear on the market and for quite some time it was assumed that they were the result of classical breeding approaches. Recently, they were shown to be genetically engineered (GE) [3,4], carrying an unspecific maize DFR under control of the *35S* promoter, to restore the ability to convert DHK into leucopelargonidin, the immediate precursor of the orange-red pelargonidin based pigments. The recall campaign for the undeclared commercial orange flowering petunias was causing economic losses to breeders and plant producers world-wide. The question of the origin of the undeclared transgenic orange petunia, and how they entered the breeding process remained open. There was broad agreement that the transgenic construct probably derived from the first scientific petunia [2], however, in the current debate it remained always unmentioned that there was a second scientific petunia [5], which was constructed with the same GE-elements, which could also have triggered the avalanche.

We evaluated three orange petunia cultivars bred in different countries. We show that from the two possible known sources indeed only the Meyer construct [2] can be responsible. Other potential, so far unknown sources can also be ruled out, because a special maize *DFR* variant is present. This includes, at the 3'-end, an additional 144 bp segment from the non-viral transposable *Cin4-1* sequence, which does not add any functional advantage with respect to DFR activity, and therefore, would most likely not have been used in the construction of a further plasmid for breeding GE-orange petunias. This unequivocally points at the first scientific GE-petunia from the 1980s as the A_1 source, which is further underpinned e.g. by the presence of specific restriction sites, parts of the untranslated sequences, and the same arrangement of the building blocks of the transformation plasmid used. A few scenarios how GE petunias could enter the breeding programs are possible. The most probable one is that over time the origin of orange petunia was forgotten and transgenic lines uniquely entered a conventional breeding program [4]. This is underpinned by the fact that, to date, only one construct was detected in all orange petunia cultivars.

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