PiggyBack™ expression, a versatile method for enhancing amino acid composition in plants:

Improved milk production in dairy cows by enriching the histidine content of canola seeds

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Abstract

In addition to the primary use of canola in oil pressing, canola is important as a feed supplement for livestock. We have developed a new approach (termed PiggyBack™ expression) that can be used to enhance the histidine content of canola protein. Using PiggyBack™ expression (a novel recombinant approach for generically improving the amino acid composition in crop plants) the histidine content in canola seeds can be substantially improved (greater than 20%). Given that histidine is the limiting amino acid in terms of milk production in grass silage fed to dairy cows, PiggyBack™-mediated enhancement of histidine content in canola has clear potential for improving milk production economy. Consistent with this view, higher milk yields from dairy cows are obtained by increasing the level of histidine in feed.

Introduction

Following the oil pressing process, the canola oil cake remaining retains a high value in terms of its use as a feed supplement for livestock. In grass silage-based diets fed to dairy cows, histidine is the limiting factor in milk production (Huhtanen*et al.*, 2001). Direct addition of histidine to feed to improve milk production is not economic and additionally rumen breakdown of added histidine is a problem. An alternative to the addition of histidine to feed would be to directly improve the histidine content of canola oil seeds. In this booklet we outline a new recombinant technology (PiggyBack™ expression) that can be used to improve the content of any desired amino acid in crop plants. We used PiggyBack™ expression to substantially enhance the histidine content in *Brassica* oil seeds.

Method: PiggyBack™ expression technology

PiggyBack™ expression technology is a novel, generic recombinant approach for improving the amino acid composition in crop plants (Wahlroos *et al.*, 2004a, Wahlroos *et al*., 2004b). To enhance the histidine content in oil seed crops the basic strategy has been to express a repeat-histidine-unit oligonucleotide cassette from a seed specific promoter and to target the expressed protein to oil bodies using the intracellular carrier protein, oleosin. The stability of the recombinant histidine-oleosin protein is assessed prior to plant transformation using an *in vitro* (cell free translation) test, which provides a reliable performance indicator of the likely stability of the recombinant protein *in vivo*. By opting to use intracellular carrier proteins other that oleosin (e.g. TMV movement protein) the amino acid targeted for improvement can be expressed throughout the whole plant, making the approach especially useful in crops whose silage is intended for use as animal feed.

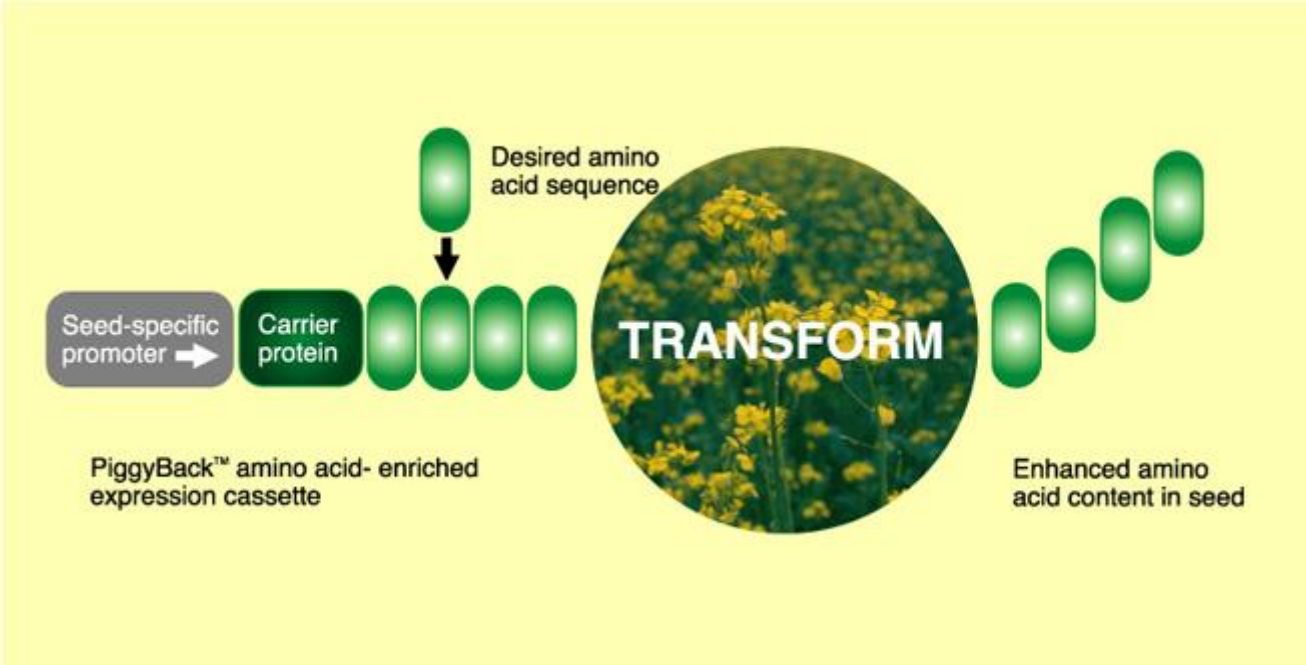

Figure 1. Schematic representation of PiggyBack™-mediated improvement of histidine content in Canola.

Results

A histidine expression cassette containing fifty-six histidine (his_{56}) units (assembled from four histidine-repeat cassettes each comprised of 14 histidine codons) was found to be optimal for stable expression in a cell free translation assay (Figure 2). Transformation of *Brassica napus* plants with the his_{56} PiggyBack™ expression cassette resulted in a notable improvement in the histidine content in transformed plants compared to control wild-type plants (Table 1).

Figure 2. Western blot analysis indicates that a histidine expression cassette with 56 repeat-histidine-codons was optimal for stable expression in an *in vitro* (cell free translation) assay.

Table 1. Improved histidine content in seeds of *Brassica napus* plants transformed with the his_{56} PiggyBack™ expression vector.

Seeds from:	**Histidine content (g/kg dry weight)**	**Increase in histidine content**
Wild-type (untransformed control) plant	7.2*	N/A
Transformed line A	8.3	15%
Transformed line B	8.8	22%
Transformed line C	8.4	16%
Transformed line D	9.0	25%

* average histidine content in seeds from four control plants

Conclusions

PiggyBack™ expression is an appropriate strategy for substantially improving the histidine content in canola oil seed; additionally, PiggyBack™ expression has major potential for the improvement of amino acid content in crop plants in general. Histidine improved canola varieties have clear application possibilities in the improvement of milk yield in dairy cows.

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