Podshatter resistance: from gene function validation in *Arabidopsis* towards a productivity trait in oilseed rape

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ABSTRACT

Brassica s form pods, which release the seed following the separation of the valves. This active process during fruit maturation is called dehiscence. There is very little genetic variability for shatter resistance within the commercial germ plasm.

Recently, several genes, which play an essential role in pod dehiscence have been identified in *Arabidopsis thaliana*. The functional analysis of these genes in pod development and their use as tools to control valve separation in *Arabidopsis* has enabled the design of approaches to engineer pod shatter resistance in oilseed rape.

We demonstrate that seed shatter can be efficiently controlled through the inhibition of the expression and/or overexpression of specific regulatory genes in *B. napus*. Our results from greenhouse and field experiments illustrate how crop-related model species, such as *Arabidopsis* for *Brassica* crops, can play a key role in discovery research towards solutions for the improvement of relevant traits.

Key words : pod shatter, *Arabidopsis*, function validation, dehiscence

INTRODUCTION

Shedding of the seed (pod shatter) before or during the crop harvest is an inherent problem in oilseed rape. Pod shatter related yield losses typically range from 10-25%, but can reach 50% depending on the weather conditions. Current commercial oilseed rape varieties are extremely susceptible to shattering and conventional breeding has not been able to bring solutions for this problem.

The relevant impact of pods shattering on seed recovery and hence on yield and on farm management practices have been endorsing the interest in a 'shatterproof' trait for oilseed rape.
Most plants have developed strategies to reproduce through the seed, which are being released from the fruit through a variety of mechanisms. Siliques or pods from the *Brassicaceae* release their seed through an active process, called dehiscence. Pod dehiscence is an orchestrated series of events resulting in the physical dissociation of the valves along the fruit. The dehiscence zone (DZ) demarkates the exact location of this dissociation.

Recently the genetic basis for pod dehiscence has been studied in detail in *Arabidopsis thaliana*, a shattering *Brassica* related species. Several regulatory genes encoding putative transcription factors were shown to be specifically involved in the molecular orchestration of the dehiscence process. These genes are encoding putative MADS box and helix-loop-helix transcription factors. It was shown that under- or overexpression of these genes can result in shatter resistant pods in *Arabidopsis thaliana* (Ferrandiz et al., 2000; Liljegren et al , 2000; Rajani et al., 2001).

This paper describes transgenic approaches to validate the potential use of these candidate genes to obtain podshatter resistance. Furthermore these tools have also been tested and validated in the target crop: oilseed rape.

MATERIALS AND METHODS

Arabidopsis thaliana and a *Brassica napus* spring cultivar have been transformed using an *Agrobacterium* Ti-plasmid mediated transformation procedure. *Brassica* transgenic lines are characterised with respect to their T-DNA integration pattern. Single copy T-DNA lines have been selected for more detailed phenotyping

The random impact test (RIT) is a quantitative assay defining pod strength at maturity. Mature pods are being shaken under reproducible conditions for defined timepoints. From these observations the 'pod half life' can be calculated, being the shaking time needed to open half of the pods.

RESULTS

The FUL gene encodes a putative MADS box transcription factor. In *Arabidopsis* FUL is encoded by a single gene. A structural knockout of the gene results in a specific silique phenotype: short siliques and in the arrest of valve expansion. It was recently shown that constitutive expression of the FUL gene in *Arabidopsis* under the control of the CaMV35S promoter results in two different types of transformants: short siliques, probably caused by co-suppression) and pod shatter resistant, probably due to enhanced expression). In addition, an early flowering phenotype has been observed as a pleiotropic effect in most of the transgenic *Arabidopsis* lines.

We have tried to exploit this concept in *Brassica napus* . To this end the *Arabidopsis* FUL gene was expressed under control of the CaMV35S promoter in a spring oilseed rape variety. More than 70 independent transgenic lines *B. napus* were generated and characterised for T-DNA integration pattern and plant phenotype. None of the transgenic lines revealed obvious aberrant phenotypic alterations during vegetative growth. Early flowering, as seen in *Arabidopsis*, was not observed in the transgenic *Brassica* lines. In a limited number of transformants (9/73) a podshatter resistant phenotype was observed on the original transformants. These lines were analysed in more detail with respect to stability of this phenotype and the morphological basis for the shatter resistance. PS^R lines were grown for 2 consecutive generations in the greenhouse. All transgenic progenies of the selected lines were shown to inherit the resistance. Microscopical analysis of immature pods of PS^R lines showed that the structure at the dehiscence zone was profoundly changed. The dehiscence zone of the pods and the external 'suture' along the pod are no longer recognisable, resulting in a tube-like structure.

Species	# Transformants	# Podshatter lines	Short siliques
Arabidopsis	154	46 (30%)	108 (70%)
Brassica	73	9 (12.5%)	0 (0%)

Table: Number of PS^R transformants generated for *Arabidopsis* & *Brassica* using the 35S-FUL (*Arabidopsis*) construct.

Figure: Phenotype of mature pods: Pods of wild type line open easily, whereas pods from a selected PS^R line do not open, they break across the pods.

The random impact test was used to assess the shatter resistance of these lines. The established RIT assay was unable to quantify pod strength in the selected PS^R lines, due to the fact that the mature pods of the selected lines do not open over the dehiscence zone at all; they break, keeping most of the seed inside.

In order to test the agronomic relevance of the obtained transgenic PS^R lines, seeds were increased on heterozygous lines and tested in the field. Plants of independent transformants were grown in a single row configuration in 2002. During vegetative growth no obvious abnormalities could be observed; at maturity, lack of dehiscence zone and pod suture were observed, confirming the greenhouse data. In order to test harvestibility of these pods plots were combined row by row. Even under stringent harvesting conditions, the harvester was unable to tresh the seed out of the pods. These data clearly indicate that the obtained podshatter resistance is too strong for current harvesting practices. Moderation of the obtained shatter resistance could either be addressed by reducing strength of the used promoter or changing its specificity.

Other genes in *Arabidopsis* thaliana were shown to impact on silique opening. SHP encodes a putative MADS box transcription factor. In *Arabidopsis*, there are two functionally redundant genes: SHP1 (AGL1) and SHP2 (AGL5). A structural knockout of either SHP1 or SHP2 does not result in a particular phenotypic alteration. A knockout of both genes together result in pod shatter resistant siliques in *Arabidopsis* (Liljegren et al., 2000). A transgenic approach, based on RNAi technology, has been developed to mimic the double SHP1/SHP2 mutant phenotype. To this end two different regions of the SHP coding sequence (of about 150bp) were defined as specific homology target region for RNAi down-regulation. RNAi constructs, driven by the 35S promoter were tested in *Arabidopsis*. More than 100 independent transgenic lines were scored for podshatter resistance. The DNA sequence located between the MADS box and the K-box was revealed to be the more relevant for obtaining podshatter resistant phenotypes. The next step will be to implement this strategy in oilseed rape.

DISCUSSION

Gene function validation experiments in *Arabidopsis* and *Brassica napus* confirm that regulatory genes are key entry steps for strategies towards controlling podshatter in *Brassica*. We have shown that the different approaches can result into shatter control with different efficacies. The obtained shatter resistant phenotypes were shown to be stable under field conditions. The agronomic relevance of these phenotypes need to be addressed in more detail.

REFERENCES

Ferrandiz, C. S. J. Liljegren and M.F. Yanofsky 2000: Negative regulation of the SHATTERPROOF genes by FRUITFULL during *Arabidopsis* fruit development. Science 289, 436-438.

Liljegren, S.J., G.S. Ditta, Y. Eshed, B. Savidge, J.L. Bowman and M.F. Yanofsky 2000 : SHATTERPROOF MADS-box genes control seed dispersal in *Arabidopsis* Nature 404: 766-770.

Rajani, S. and V. Sundaresan, 2001 : The *Arabidopsis* myc/bHLH gene ALCATRAZ enables cell separation in fruit dehiscence. Current Biology 11, 1914-1922