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Pod Shattering Resistance in Canola Brassica napus Mediated Gene Transfer

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V- SUMMARY AND RECOMMENDATIONS

Pod shattering after maturity is a major problem in canola production around the world, and this can lead to a loss of up to 50% of yield if harvest is delayed. In the recent period, some researchers have sought to use genetic engineering techniques to transfer pod shattering resistance as a promising alternative approach to overcome the problems of hybridization.

The present study was carried out in Genetics Department, Faculty of Agriculture, Fayoum University, Egypt, during the period from 2016 to 2018. The rapeseed (*Brassica juncea*) accessions numbers PI 649105 and PI 271442 were used as a source of pod shattering resistance genes. The spring oilseed rape (*Brassica napus*L.) cultivar Serw 4 was used as a source of explants for agrobacterium-mediated genetic transformation,

In this study, genomic DNA was extracted from the rapeseed (*B.juncea*) and *B.napus*. The specific primers FU 1, FU 2, FU 3, Ful 2 and Ful 3which corresponded to pod shattering resistance were used to amplify *SHAT 1, SHAT2, SHAT 3, FUL 2 and FUL 3*genes, respectively by using

polymerase chain reaction (PCR).The PCR products were sequenced andsubjected to alignment with sequences of the GenBank, EMBL, DDBJ and PDB from Brassica using the BLASTN 2.2.18; the SIB Bioinformatics Resource Portal (ExPASy);the BLASTP 2.2.18and predicted protein sub-cellular localization using LocTree3.

E. coli DH5α, JM 109 and TOP10 were used in all the molecular biological experiments and *Agrobacterium tumefaciens*LBA4404 was used for plant transformation. The RBCT&A Vector System was used for sequencing PCR products and the plant binary vectors pCAMBIA1301 was used for transformation.Cotyledons, hypocotyls and cotyledon petiolsfrom Serw 4 cultivar were infected with agrobacterium.

The results of the present study are summarized as follows:

PCR analysis:

1. No bands are shown when using the five primerpairs to amplify a specific part of the *SHAT* 1-3 and *FUL* 2&3 genes of the *B.napus* cultivar Serw 4.
2. No banding patterns are shown when FU 1 and Ful 3 primer pairs used to amplify a specific part of the *SHAT* 1 and *FUL*3 genes of *B.juncea*.

3. Threeprimerpairs FU 2, FU 3 and Ful2showed one sharp band with a molecular weight of 750 and 900 and 1200 bpfor *SHAT* 2, *SHAT* 3 and *FUL* 2genes, respectively in each of the two *B.juncea* accessions.

Gene cloning and DNA sequence analysis

1. A-Tailing overhangs *SHAT* 2, *SHAT* 3 or *FUL* 2 products were cloned into RBC T&A cloning vector resulting RBC- *SHAT* 2, - *SHAT* 3 or -*FUL*2.
2. *E.coli* competent cells were transformed with RBC- *SHAT* 2, - *SHAT* 3 or -*FUL* 2and sequenced.
3. Results of sequence analysis using BLASTN software showed a 98% similarity and 93% query covered between *B.junceaSHAT* 2 gene with *B.rapa*subsppekinensis; *SHAT* 3 gene failed in sequencing; 97% similarity and 14% query covered between the *B.junceaFUL* 2 gene with *B.rapa* subsp. pekinensis too.
4. Also, sequence analysis showed a 100% similarity between the two *B.juncea*sequences for *SHAT* 2 gene.
5. The genetic relationship of *SHAT* 2 gene based on nucleotide sequence using UPGMA revealed that this gene was farther apart from *B. rapa*.

6. Translation results of EXPASY showed one open reading frameof the protein produced from *SHAT*2 sequence.A 5'3' frame 1 was selected to predict the function of the gene product, showing its compatibility with the off-cellular region GO0005576.
7. Results of computer analysis using BLASTP software showed that a 94% similarity between *SHAT* 2 protein with En/Spm-related transposon protein andthe UPGEMA tree showed the *SHAT* 2 ORF closely related with En/Spm-related transposon protein (ACG60686). So, we used *SHAT* 2 gene in Agrobacterium transformation.

Gene cloning and Agrobacterium transformation

1. The *SHAT* 2-Hind III/Bam HI gene was amplified and cloned into the Hind III/Bam HI-digested pCAMBIA 1301 resulting in pCAMBIA 1301- *SHAT* 2.
2. *A.tumefaciens*LBA4404 wastransformed with pCAMBIA 1301- *SHAT* 2 and can be ready to plant transformation.

B.napus genetic transformation

1. Cotyledon, hypocotyl and cotyledon petiol explants of *B.napus* cultivar Serw 4 were transformed.Multiple shoot derived from each explants were selected on MS medium

containing Kanamycin(25µg/ml) and Carbenicillin(500µg/ml).

2. High frequency shoot regeneration was obtained with hypocotyl explants on basal MS salts supplemented withoptimized hormone concentrations for callus induction, shoot initiation, shoot outgrowthand root initiation.
3. Transformed regenerated plants were confirmed by PCR using specifically designed primers. The results showed the integration of *SHAT* 2 gene into *B.napus* genome.

Recommendations

1. We didn't only discover a novel gene product but also provided usefulinformation about extra-cellular protein.
2. As a result, one type of pCAMBIA-*SHAT* 2 expression vectorwas constructed.
3. Results indicated that Agrobacterium-mediated transformation was successful for pod shattering resistance gene.
4. In the future, the expression of these genes will be studied in the transgenic plants to confirm the stability of pod shattering resistance.