Research Article

Expression Pattern Analysis of Core Regulatory Module *SHPs-FUL* Transcripts in Rapeseed Pod Shattering

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Abstract | Non-synchronous pod shattering is the main cause of yield losses in canola. The expression of *SHATTERPROOF1/2* (*SHP1*and *SHP2*) and FRUITFULL (FUL) MADS-box genes is fundamental to fruit dehiscence zone and valve margin, respectively. The present study was envisaged to isolate the orthologs of *SHPs* and *FUL* from local canola "Pakola" and "Punjab Sarsoon 3", and to study expression patterns of their transcripts. Morphological data revealed significant difference between pod wall thicknesses, seeds per pod and pod length between the two cultivars. PCR amplification and sequencing revealed that two products namely *BnSHP1-like* and *BnSHP2-like* could be identified. The sequence analysis of *BnSHP1-like* and *BnSHP2-like* demonstrated that these genes are 747 bp and 735 bp in size, respectively. The nucleotide alignments revealed 98% identity of *BnSHP1-like* and *BnSHP2-like* with *BnSHP1* and *BnSHP2* sequences. The sequence homology was estimated to be 95 and 96% at amino acid level for *BnSHP1-like* and *BnSHP2-like* genes, respectively. The phylogenetic reconstruction of *SHP1* and *SHP2* homologs from other species conglomerated *BnSHP1-like* and *BnSHP2-like* into their respective clades. Semi-quantitative RT-PCR revealed overlapping expression of both the *BnSHP1-like* and *BnSHP2-like* transcripts in flower and siliques while no expression in the leaf tissues was observed. A strong expression for *FUL* gene was detectable in mature silique and silique from upper portion of plant as compared to other tissues of "Pakola" and "Punjab Sarsoon 3". Our results flaunt basic gene expression information about shattering genes for developing genome edited plants to prevent yield losses in canola in future.

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Introduction

Brassica comprising about 40 species is commercially an important genus with respect to oil, seed, vegetable, forage and condiment production. *Brassica* oilseeds are well entrenched in the cropping system of world and reduce the gap between yield quantity, production and consumption; if made competitive with other field crops (Canola Council of Canada, 1995). Canola (*Brassica napus* L.), 2n=46=38, genome AnAnCnCn) is an amphidiploid plant of the eudicot angiosperms that arose 10,000 years before in consequence of hybridization of turnip rape (*Brassica rapa* L.; genome ArAr, 2n=26= 20) and cabbage (*Brassica*

oleracea L.; genome CuCo, 2n=26= 18), followed by duplication of chromosome (U, 1935). Globally, canola is considered as the third most significant oilseed crop (Basalma, 2008). However, premature and unsynchronized pod shattering i.e. dehiscence inflicts heavy losses in crop yield (Raman et al., 2011). Twenty to fifty percent of total yield is lost during harvesting and crushing as the pods rupture and discharge the seeds before the farmer can harvest it (Price et al., 1996; MacLeod, 1981; Child et al., 2003). This unsynchronized pod shattering along with biotic and abiotic stress is one of the major issues for loss of canola yield and production. Commercially, early and uncoordinated pod shattering results in significant pre-harvest losses and therefore considerably reduces the net yield. Resistance to shattering is a desirable trait for rapeseed improvement (Kadkol, 2009). But efforts to control pre-mature pod shattering remained fragmented. Even interspecific hybridization using *B. nigra*, *B. juncea* and *B. rapa* is not totally successful due to integration of other undesirable characters (Prakash and Chopra, 1990). Therefore, need for genetic improvement of this crop with modern genetic engineering for synchronous opening of pods is inevitable.

Out of three important parts of *Arabidopsis* silique, valve elongation and differentiation is under the control of Fruitfull (FUL) (Ferrándiz et al., 2000; Liljegren et al., 2004). This gene is recruited in antagonizing the Shatterproof1/2 (SHP1/SHP2) and another gene Indehiscent (IND) at the dehiscence zone (DZ) (Liljegren et al., 2000; Theissen, 2000). At this part these three genes control normal lignification. Both genes redundantly control the valve margin and dehiscence zones in the fruit. When these two effective genes are mutated, the seed pod is unable to shatter. During the flower and pod development these genes are also expressed in inflorescence and fruiting body of *Arabidopsis* plant (Colombo et al., 2009). Single mutants of *SHP1* and *SHP2* genes are indication for a wild type phenotype, but the *SHP1* and *SHP2* double mutant has fruits which do not release seeds, signifying that both seed dehiscence in *Arabidopsis* is controlled by the together effect of both genes. The SHP1/2 and IND expression is also repressed by Replumless (RPL) which is involved in replum formation (Roeder et al., 2003); the third important part of the silique. FUL is indirectly implicated in controlling two other transcription factors Alcatraz (ALC) and Spatula (SPT). These genes confer formation of the separation layer (Rajani and Sundaresan, 2001). FUL and SHP1/2 TFs belonging to Agamous clade of the MADS-box family constitute the core regulatory module SHP-FUL (Dong and Wang, 2015). These proteins have four domains; M, for binding the DNA; I is a variable region; the K domain is recruited in activation and C terminal is the longest region with interaction properties (Schwarz-Sommer et al., 1990; Becker and Theissen, 2003). The most desirable solution to shattering problem of canola is to delay pod shattering by knocking out SHP genes and activating the expression of FUL until the susceptible crop is ready for harvesting. But before developing the genome edited plants it is imperative to study these genes elaborately in local canola. Therefore, this research activity was envisaged with the objectives; 1) to isolate these genes from local canola cultivars; 2) to study their expression patterns; and 3) to unravel their evolutionary relationship with other genes. The isolation of SHP genes and expression patterns of SHP-FUL provides the essential genetic understanding to design new genome editing strategy for breeding against non-synchronous seed shattering.

Materials and Methods

Varieties selected and morphological studies

Seeds of canola (*Brassica napus* L.) cultivars "Pakola" (less shattering susceptible) and "Punjab Sarsoon 3" (shattering susceptible) were obtained from National Oilseed Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan. Plants were grown in pots at 22°C in the glass house and open fields as well. Mature pods were harvested for morphological studies. Mature pods or siliques of both the varieties were harvested for screening of morphological characters such as pods length, pods weight, seeds per pod and wall thickness of pods with respect to shattering of crop. Pod wall thickness was measured with venire caliper and pod weight was measured with electric balance.

The plant material was harvested at vegetative, flowering and fruiting stages and stocked at -80 °C for nucleic acid extraction.

Designing of primers for Shatterproof 1/2 and Fruitfull genes

Nucleotide sequences of *Shatterproof1/2* (*SHP1/2*) and *Fruitfull* (*FUL*) were retrieved from National Center for Biotechnology Information (NCBI) database. The primers were designed from the

coding region of *SHP1/2* genes of *Brassica napus* (*BnSHP1* with accession No. AY036062; *BnSHP2* accession No. EU424343; *BnFUL* accession No. DQ414534.1) with the help of MacVector™ 7.2.3 software (Accelrys Inc.; gcg/Wisconsin Package, University of Wisconsin). Primer sequences used for gene amplification and expression analyses are listed in Table 1.

Table 1: *List of primers used for isolation and expression analysis of SHP1/2-like FUL transcripts from local canola cultivars.*

Gene amplified	Sequence (5' to 3')
For gene amplification with PCR	
Bn-SHP1	ATGGAGGAAGGTGGGAGTAGTCAC, TTACACAAGTTGAAGAGGAGGTTG
Bn-SHP2	ATGGAGGGTGGTGCGATGAATGAG, AATCAAACAAGTTGCAGAGGTAGG
Bn-FULa	GGTTCAGCTGAAGAGGATAG, AAGTACCTCAACTCTTGCCT
For expression analysis with semi-quantitative RT-PCR and real-time RT-PCR	
SHP1-like	ATGGAGGAAGGTGGGAGTAGTCAC, GATGACGAGGGCAACTTCGGCATC
SHP2-like	CTCGAGTGGAGAAGATGACAAGAG,ATGGAGGGTGGTGCGATGAATGAG
Bn-FULc	CAAAGAGAAGGTCTGGTTTG, CACTTTGTGAAATGTCTCGG

Total RNA extraction and first strand cDNA synthesis

Total RNA from leaves, inflorescence and pods of the canola plants was extracted using PureLink™ RNA Mini kit (Invitrogen). Quantification of RNA was determined with thermo Scientific's NanoDrop™ Lite and quality of extracted RNA was checked on 1.5% agarose gel. RevertAid™ reverse transcriptase Enzyme (Fermentas™ Cat. No. K1621) was used to synthesis first strand cDNA.

Isolation of SHP1/2 homologs from canola

Standard PCR was performed for the amplification of *SHP1/2* homologs from *Brassica napus* cultivars "Pakola". The PCR profile was an initial denaturation step at 95°C for 5 minutes, followed by 37 cycles of 94°C for 1 minute, 58°C for 1 minute and 68°C for 1 minute, and a post extension temperature of 68°C for 10 minutes. PCR ampliconss were analyzed on 2% high resolution agarose gel and photographed. The expected size fragments were cut with scalpel under UV light and purified using PCR GeneJET PCR Purification Kit (K0701). The purified PCR products were sequenced by MACROGEN (Korea).

Sequence analysis and phylogenetic reconstruction

Newly isolated sequences were edited using MacVector 7.2 software. These sequences were assembled using Assembly Align program. Sequences were annotated and mutations in different domains of new homologs were documented. ClustalW alignments were made to compare the newly isolated sequences from Pakola with previously isolated *Brassica napus SHP1/2*. Both the nucleotide and amino acid alignments were generated for comparison.

In order to infer the evolutionary relationship of *SHP1/2* genes in different species, phylogenetic analysis was carried out. For this purpose, coding sequences of *SHP1/2* genes from different species including *Brassica napus, Arabidopsis thaliana*, *Hordeum vulgare* and *Arabidopsis lyrata* were collected. A neighbor joining tree in MEGA6 software was constructed with P uncorrected distance. To validate the reliability of tree, 1000 replications of bootstraps were calculated. Similarly, a phylogenetic tree was also generated for *FUL* gene homologs.

Gene expression analysis

Multiplex semi-quantitative RT-PCR was executed to differentiate the expression patterns of newly extracted *BnSHP1/2-like* sequence in leaves, inflorescence and pods of the canola. The reaction was carried out as described (Khan et al., 2013). For endogenous reference *18SrRNA* gene was used. The reaction was carried out in biological and technical replicates. The amplified transcripts were analyzed on 2% agarose gel and photographed.

Real time PCR was performed to differentiate the expression profiles of *FUL* gene sequences in leaves, flower bud, mature flower, silique bud, immature silique, mature silique, silique from lower portion of plant, silique from upper portion of plant of Pakola and Punjab Sarsoon 3. For endogenous control *18SrRNA* was employed. The reaction was carried out in biological and technical replicates.

Statistical analysis

Data was collected on weight, length and seed no. per pod, were analyzed statistically by using ANOVA technique and LSD test at probability level of 5 % to compare the significance among parameters means (Steel et al., 1997).

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Results and Discussion

Pod morphology is correlated with shattering

The pods were characterized on the basis of mature pods,pod wall thickness, weight per pod, pod length and seeds per pod of "Pakola" (less shattering susceptible) and "Punjab Sarsoon 3" (shattering susceptible). The results revealed significant differences in pod wall thickness, pod length and seeds per pod (p value <0.05) between the two cultivars (Table 2). Thus, degree of pod shattering susceptibility has some relationship with pod morphology.

Table 2: *Morphological characterization of oilseed rape pods.*

Parameters	Pakola	Punjab Sarsoon 3	P value
Pod wall thickness (cm)	0.1*	0.08 *	<0.05
Pod length (cm)	5.7*	5.9 *	<0.05
Pod weight (g)	0.54	0.51	> 0.05
Seeds per pod (Nr.)	24*	22 *	<0.05

**Marks significant difference (p<0.05).*

BnSHP1/2-like homologs from Pakola cultivar can be isolated

In order to isolate *BnSHP1/2-like* sequences, total RNA was extracted from leaf, flower and pods of Pakola cultivar of canola. Taking cDNA as template gene specific primers were used to amplify coding regions of the *BnSHP1/2-like* homologs. The PCR result exhibited that two products could be identified. A strong band for *BnSHP1-like* was detectable in case of flower and pod tissues while in leaf no amplification was visible (Figure 1). Similarly, *BnSHP2-like* gene was also amplified in the flower and pods tissues. These PCR products were cut from the agarose gel, purified and sequenced. Editing of sequences was done using Mac Vector™ 7.2.3. (Accerlrys Inc.) gcg/Wisconsin package university of Wisconsin) software. Blast*n* results revealed maximum homology with *SHP1* and *SHP2* genes of *B. napus* followed by *A. thaliana* thereby confirming the presence of two novel sequences of *BnSHP1-like* and *BnSHP2-like* genes in canola.

The C-terminal region of BnSHP1/2-like is variable

The *BnSHP1/2-like* sequence analyses revealed no significant variations in the length of the nucleotide sequences (Figure 2). The coding sequence length was limited to 747 bp and 735 bp for *BnSHP1-like* and *BnSHP2-like* genes, respectively as previously reported. The ClustalW alignment of nucleotide sequence of *BnSHP1/2-like* coding sequence exhibited 98% identity with previously isolated gene. Nevertheless, sequence identity was reduced to 95% at amino acid level. Merely substitution mutations in the sequences were observed, without any deletions or insertions of nucleotides. Therefore, the size of genes remained unchanged. Only 10 substitution mutations were detected in *BnSHP1-like* nucleotides sequence. All the mutations belong to non-synonymous category i.e. changing in protein sequence occurred as revealed by translated sequence. In case of *BnSHP2-like* sequence 98% identity was observed at nucleotide level, which reduced to 96 % at amino acid level. Like *BnSHP2-like*, all the 8 mutations were of non-synonymous type (Figure 2). When *BnSHP1-like* and *BnSHP2-like* compared at both the nucleotide and amino acid level, two separate cluster of genes were prominently detectable (Figures 2 and 3).

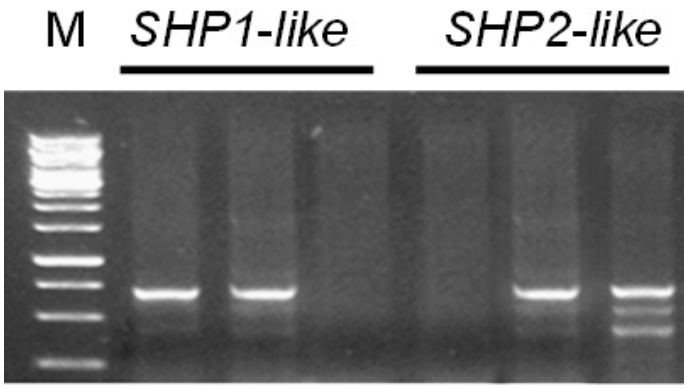

Figure 1: Amplification of *Shatterproof 1/2* genes from local canola cultivars: *Gel picture showing the PCR amplification of BnSHP1-like and BnSHP2-like transcripts from flower and pod tissues of Brassica napus (Pakola); F1 (Flower BnSHP1-like); F2 (Flower BnSHP2-like); L1 (Leaf BnSHP1-like); L2 (Leaf BnSHP2-like); S1 (Silique BnSHP1-like); S2 (Silique BnSHP2-like); M (1kb ladder).*

As BnSHP1/2-like genes belong to MADS-box family of transcription factors therefore, they can be unequivocally separated into 4 distinct domains. The first 60 aa belong to MADS-domain (M-domain), which is a DNA binding domain, followed by the I region, K-box and C-terminal region. Figure 3C demonstrates that there were no variations in BnSHP2-like proteins in the M-domain but surprisingly two non-synonymous mutations occurred in this domain, which is supposed to be highly conserved in the MADS-box family. The I-region and the K-box seemed to be conserved but the most dramatic changes have occurred in the C-terminal region. What made it different from

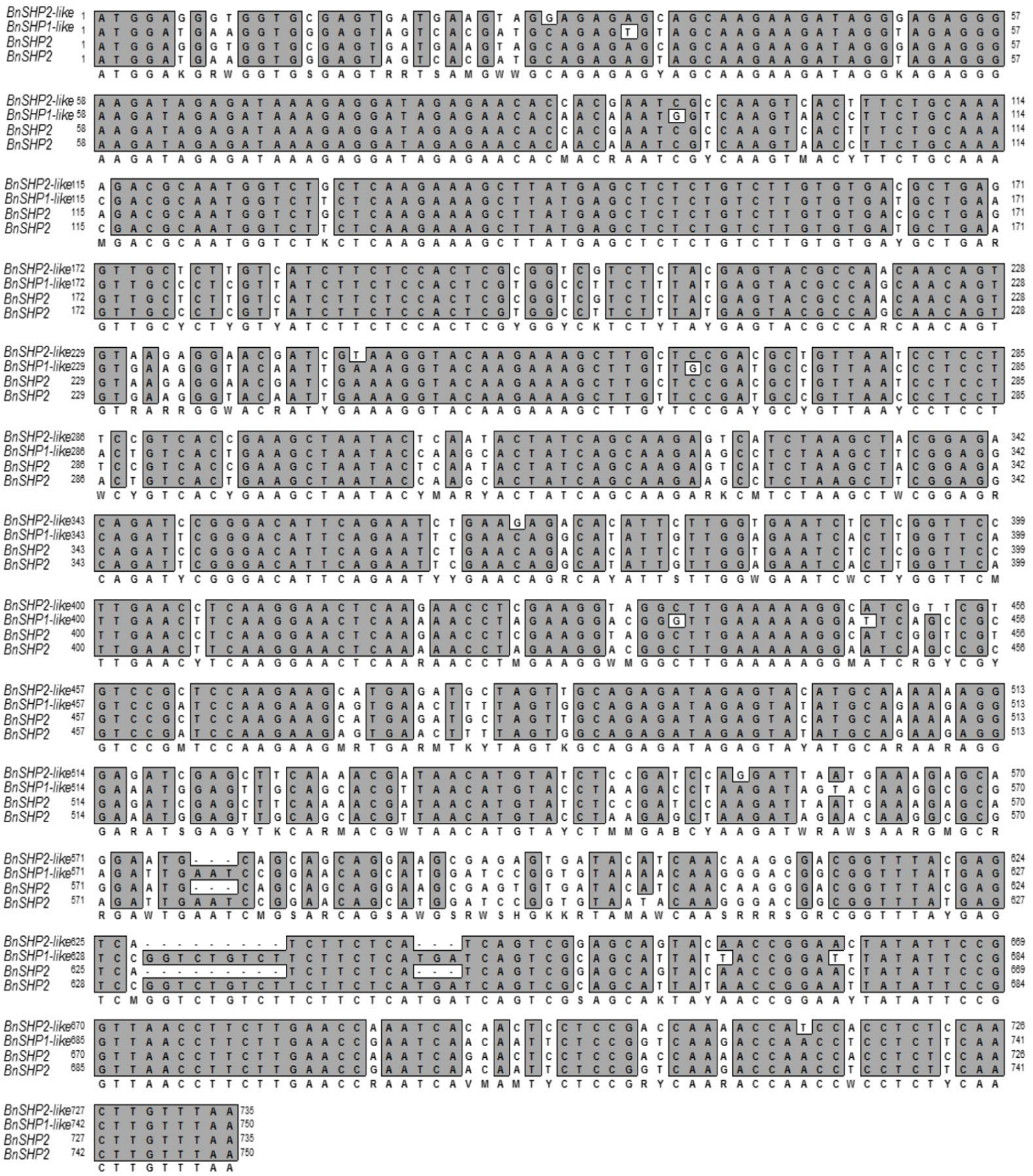

Figure 2: Sequence analysis of *BnSHP1/2-like* genes: *ClustalW multiple alignment of newly isolated BnSHP1-like, BnSHP2-like, BnSHP1 and BnSHP2 nucleotide sequences with Mac Vector™ 7.2.3. (Accerlrys Inc.) gcg/Wisconsin package university of Wisconsin). Nucleotides in block highlight similarity.*

other domains is the presence of 5 and 3 substitutions in case of BnSHP1-like and BnSHP2-like proteins, respectively. This region is usually involved in protein-protein interactions (Egea-Cortines et al., 1999).

From the above results it can be inferred that two new *BnSHP1-like* and *BnSHP2-like* sequences exist in "Pakola" variety of canola. As these sequences have higher homology with previously isolated genes, therefore they might be the alleles rather than new genes. Nevertheless, *BnSHP1/2-like* genes have variable C-terminal region, which is site for

Figure 3: C-terminal of BnSHP1/2-like is variable: A) *ClustalW alignment of BnSHP1-like and BnSHP1 amino acids with Mac Vector™ 7.2.3. (Accerlrys Inc.) gcg/Wisconsin package university of Wisconsin). Amino acids in block highlight similarity;* **B)** *ClustalW alignment of BnSHP2-like and BnSHP2 amino-acids with Mac Vector™ 7.2.3. (Accerlrys Inc.) gcg/Wisconsin package university of Wisconsin). Amino acids in block show conservation;* **C)** *ClustalW multiple alignment of BnSHP1-like and BnSHP2-like with previously BnSHP1 and BnSHP2 amino-acids with Mac Vector™ 7.2.3. (Accerlrys Inc.) gcg/Wisconsin package university of Wisconsin). Amino acids in block highlight similarity. Solid block in red encloses the variable C-terminal region.*

protein-protein interactions. Hence, SHP-like proteins might change the functions of other protein by making complexes. Therefore, functional analyses are indispensable to clarify the gene/allele status of the *BnSHP1/2-like* sequences.

Phylogenetic reconstruction of BnSHP1/2-like and FUL genes

With the aim of exploring the evolutionary relationship between *BnSHP1/2-like* orthologs of various species, phylogenetic reconstruction was carried out. Figure 4A

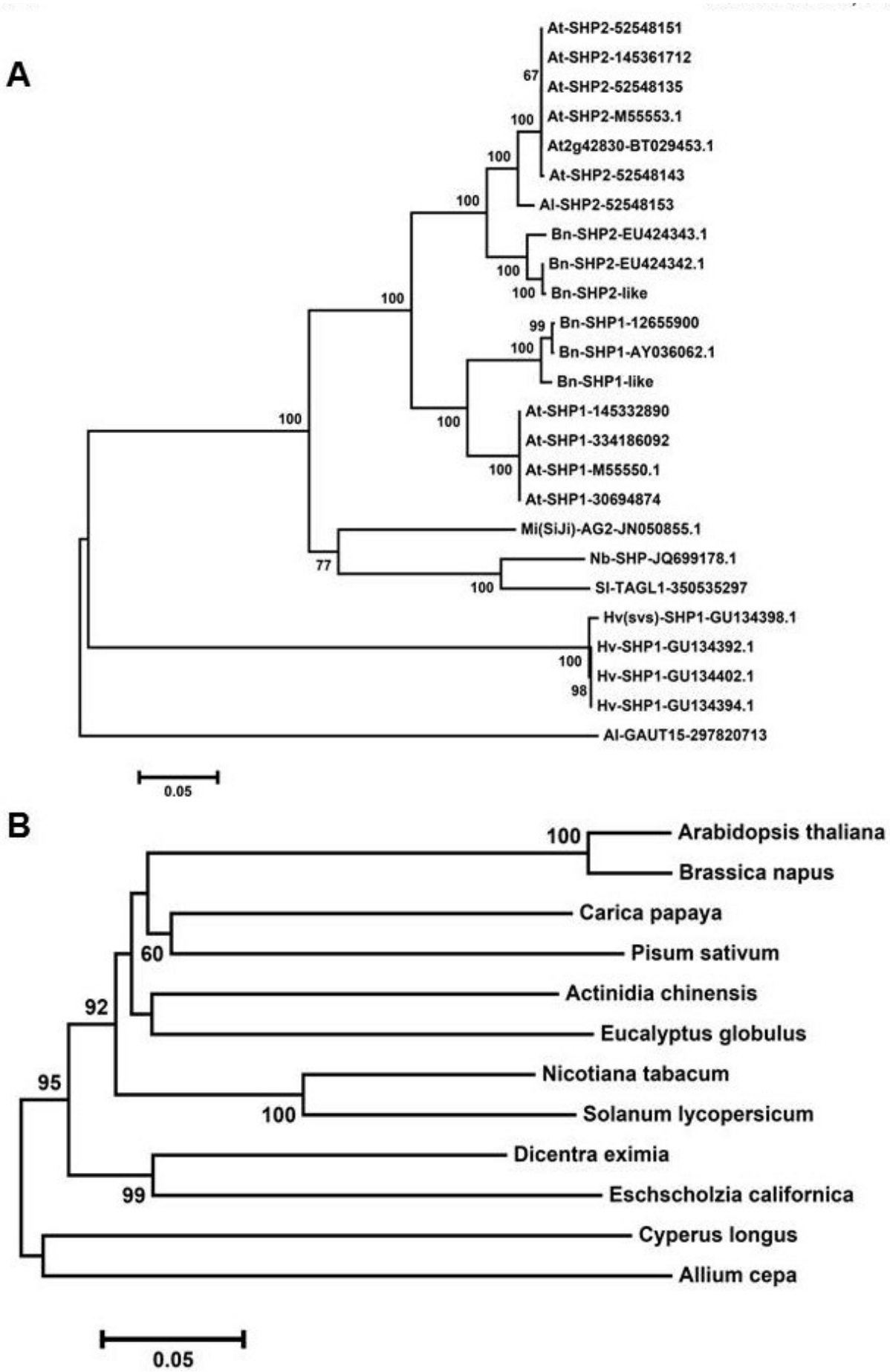

Figure 4: Phylogenetic reconstructions of *SHP1/2* and *FUL* genes: A): *A neighbor joining tree with default P uncorrected distance is constructed using MEGA6 software. Values on the nodes indicate the bootstrap replications of 1000;* **B):** *Neighbor joining tree was built using MEGA6 software. Values present on the nodes designate the bootstrap replication of 1000.*

reveals that sequences are assorted into three varied clades. Three groups are differentiated into Brassicaeceous *SHP1*, S*HP2* and monocots. The unrooted tree noticeably separates out *SHP1* and *SHP2* genes into their respective clades. Both *Bn*SHP1-*like* and *Bn*SHP2-*like* cluster with their respective *Bn*SHP1 and *Bn*SHP2 genes. This indicates divergence in sequences. Nevertheless, both the *BnSHP1-like* and *BnSHP2-like* are clustered in the Brassicaceous plants i.e. *Brassica* and *Arabidopsis*. The monocot *Hordeum vulgare* is present at the base. The reliability of the tree was indicated by higher bootstrap values. Similarly, NJ tree of *FUL* homologs also showed clear clustering into different clades (Figure 4B).

These results demonstrated that *SHP1/2* and *FUL* genes are well conserved in the different species. The newly isolated *BnSHP1/2-like* sequences though form cluster s with *Brassica* genes, nevertheless, sequence divergence does exist particularly in case of *BnSHP2-like* genes. The C-terminal domain is the least conserved region, consists mainly of hydrophobic amino acids, and plays an important role in the formation and transcriptional activation of protein complexes (Riechmann et al., 1996; Fan et al., 1997; Homa and Goto, 2001). Hence, these mutations might contribute to functional divergence. For this purpose, it is necessary to functionally characterize by site directed mutagenesis and transformation. But before transformation it was most important to perform the expression analysis of the *BnSHP1/2-like* and *FUL* genes in the different tissue of the Pakola and Punjab Sarsoon 3 varieties.

BnSHP1/2-like and FUL genes are differentially expressed in canola tissues

Previously it was demonstrated that both the *SHP1/2* genes are expressed overlapping in the valve margins of the pods. If the expression of these genes in blocked, the synchronous opening of the pods occurs (Liljegren et al., 2004; Liljegren et al., 2000). In order to find out whether any expression deviation of *BnSHP1/2-like* from *SHP1/2* genes exists, semi-quantitative RT-PCR analysis was carried out with gene specific primers. Figure 5A, B demonstrate that the transcript signals of *BnSHP1/2-like* are strong in the flower and pods tissues. But these signals are totally absent in the leaf tissues. Hence, expression divergence in different tissues is quite prominent. Nevertheless, analogous expression of both the genes in flower and pods is surprising. Silique is the tissue where *SHP1/2* genes are normally expressed in *Arabidopsis* as this is site for shattering. In case of *FUL* gene real-time PCR analysis revealed that transcript signals are strongly detectable in the mature silique, silique from lower portion of plant and from upper portion of plant from both the varieties (Figure 5C). No big difference in expression was evident between the varieties. Nevertheless, Pakola exhibited a little bit stronger expression than Punjab Sarsoon 3 in all the tissues tested.

Accretive data from sequence analysis, phylogenetic reconstruction and RT-PCR implies that *BnSHP1/2-like* sequences though conserved, exhibit variations in the gene structure and evolutionary associations with their homologs. Moreover, these genes show overlapping expression patterns in different tissues of canola.

The yield of canola- an important oilseed crop- is severely affected by pre-mature shattering of pods. The best solution to this problem is to genetically modify this crop as very less variations for crossing exist in canola due to loss in domestication process (Raman et al., 2014). Recently, evolution and expression analysis of MADS-box TF family in canola was unveiled by Wu et al. (2018). They observed the expression divergence of various shattering cascade genes in canola. Previously, it was demonstrated that Shatterproof1/2 and genes belonging to MADS-box family are recruited in controlling this problem (Becker and Theissen, 2003). SHP1 and SHP2 genes are implicated in silique opening in *Brassica* plants in case of weak expression (Ferrándiz et al., 2000; Liljegren et al., 2000; Meakin and Roberts, 1990; Wu et al., 2006). These genes act redundantly at the top of the genetic cascade that directs the development of dehiscent zone for pod dehiscence (Ferrandiz, 2002; Mongkolporn et al., 2003). Besides controlling the valve margin, FUL this gene also negatively regulates almost all the shattering cascade genes (Dong and Wang, 2015; Ferrandiz, 2002). Considering the fundamental importance of SHP-FUL genes in un-synchronized opening we set out to isolate these sequences from local cultivar "Pakola".

Interestingly two new sequences i.e. *BnSHP1/2-like* were isolated. These sequences are quite divergent from previously isolated sequences of *Brassica BnSHP1/2* and *Arabidopsis AtSHP1/2*. The size of *BnSHP1/2-like* remained conserved. The reason behind the

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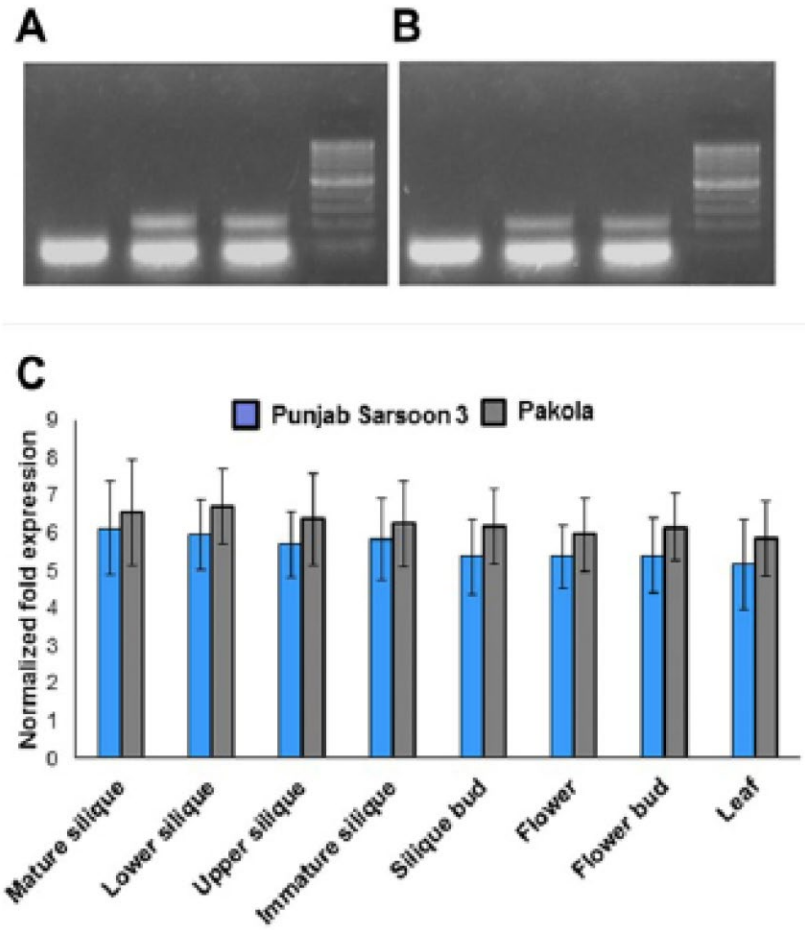

Figure 5: Expression analyses of *BnSHP1/2-like* FUL transcripts in canola: A): *BnSHP1-like transcript amplifications in flower and pod of Brassica napus (Pakola) through RT-PCR is shown. M;100 bp ladder;* **B):** *BnSHP2-like transcript amplifications in flower and pods of Brassica napus (Pakola) through RT-PCR. M;100 bp ladder;* **C):** *Normalized fold expression of FUL gene in selected tissues of Pakola (less shattering susceptible) and Punjab Sarsoon3 (shattering susceptible) through real-time RT-PCR. Error bars indicate standard deviation of the mean.*

size conservation is the absence of any deletion and insertion. Only substitutions are observed, and these are nonsynonymous because there is a change in the protein sequence after translation. Remarkably, the C-terminal is the most divergent region though mutations are present even in the M-domain which is considered to be the most conserved DNA binding region. The presence of mutations in the C-terminal region indicates that this protein might interact with other proteins for specific functions. One of the eminent characteristics of these MADS-box genes is that they show divergence in C-terminal, but it is limited to substitutions only. Generally, the C-terminal region of MADS-domain proteins is associated with transcriptional activation or the configuration of multimeric transcriptional factor complexes (Egea-Cortines et al., 1999; Cho et al., 1999; Xiao-Li et al., 2009). One of the candidates is the *FUL* gene (Ferrándiz et al., 2000). Over-expression of *FUL* gene suppresses the expression of *SHP1* and *SHP2* in *Arabidopsis* which in turn inhibits the non-synchronous opening of the siliques. Another important consideration is that expression of *BnSHP1-like* and *BnSHP2-like* is redundant. They are equally and strongly expressed in pods. However, their very high expression in flower is unexpected. Similarly, FUL transcripts are equally detected in both the less shattering susceptible and shattering susceptible cultivars. But in case of *FUL* gene, expression in flower especially carpel is natural (Gu et al., 1998). In order to have a complete picture of genetic basis of shattering mechanism in oilseed rape a comprehensive analysis including cellular expression through in situ hybridization and protein-protein interaction studies through yeast two hybrid/ Split-YFP is necessary. The expression is generally governed by promoter sequences. The redundant expressions of *BnSHP1/2-like* also indicate that their promoter sequences might be highly conserved. It is assumed that SHP-FUL module remained conserved in dry fruit species and it can be assumed that genetic interactions between SHP and FUL might be established prior to the split of rosids and asterids (Dong and Wang, 2015).

Using heterologous systems AtFUL has been transformed in *B. juncea* (Ostergaard et al., 2006) and SHP down regulated through RNAi in oilseed rape (Kord et al., 2015) but these efforts will not fruitful in true sense until all the genes involved in shattering cascade are optimized and pyramided for optimum expression in a single genetic manipulation. In these scenarios, CRISPR (Sander and Joung, 2014) can offer a useful tool for genome editing of oilseed rape for eradication of shattering problem from the base.

Conclusions and Recommendations

In conclusion, two new sequences of *BnSHP1-like* and *BnSHP2-like* genes exist in the *Brassica* local cultivar. Their sequences are conserved across Brassicacae family. Their redundant expression in the pod might indicate their functional analogy through multimeric complexes. This is supported by their diverged C-terminal region which is generally recruited in protein interactions.

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Novelty Statement

This study unveils the isolation of two important shattering genes designated as BnSHATTERPROOF 1 and BnSHATTERPROOF2 from two local cultivars of Pakistan. Besides, the expression analysis of SHP1, 2 along with another vital gene FRUITFUL have also been done, which makes a core-regulatory module of MADS-box genes recruited in shattering pathway.

Author's Contribution

MRK conceived the idea. MY, RS, MR, MA, WJ, OUR and NR did the experimental work. MY, RS, MRK and GMA analyzed the results. MY, RS, MR and MRK drafted the manuscript.

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