



Full Length Article

Ectopic Expression of Fiber Related *Gbwri1* Complements Seed Phenotype in *Arabidopsis thaliana*

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Abstract

WRINKLED1 belongs to AP2/EREB family of transcription factors whose role has been well established in seed oil biosynthesis. The objective of the study was to trace the role of fiber related *Gbwri1* in seed development and fatty acid biosynthesis. In this study, we isolated a transcript from elite fiber producing cotton (*Gossypium barbadense*), which is over-expressed in *G. barbadense* fibers as compared to *G. hirsutum* and *G. arboreum*. The putative protein encoded by this transcript exhibited homology in specific domains and protein structure with WRINKLED1 of *Arabidopsis thaliana* and was thus designated as *Gbwri1*. In this study, we investigated the functional homology of fiber elongation related *Gbwri1* with fatty acid biosynthesis regulator *Atwri1*. Ectopic expression of *Gbwri1* in *wri1-3* mutant of *A. thaliana* was analyzed. In the transgenic lines of *A. thaliana*, *Gbwri1* resumed the seed weight, seed area, and surface morphology to the wild type. *Gbwri1* transformation rescued the wrinkled phenotype of *wri1-3* mutants by resuming the expression of fatty acid biosynthesis genes biotin carboxyl carrier protein isoform 2 (*bccp2*) and keto-ACP synthase 1 (*kas1*). Moreover, the seedling development of transgenic lines on non-sucrose medium demonstrated that the *Gbwri1* was able to regulate the supply of sucrose for normal seedling establishment. Our results showed that the transformation of *Gbwri1* in *A. thaliana* *wri1-3* mutant was able to complement *wri1-3* impaired phenotype. Thus, *Gbwri1* is involved in cotton fiber development and fatty acid biosynthesis in seeds. © 2021 Friends Science Publishers

Keywords: *Gossypium barbadense*; Transcription factor; Ectopic expression; Fatty acid biosynthesis

Introduction

Oil seed crops play important role in agricultural economy next to cereals. Cotton being the most important oil seed crop is considered as valuable resource for bioenergy (Li *et al.* 2016). Identification and manipulation of the genetic factors and molecular networks controlling the fatty acid synthesis may result in significant increase in oil yield (Li-Beisson *et al.* 2013). The biosynthesis of fatty acids is a multistep process and not only involves series of genes but also highly related to other physiological processes like glycolysis, oxidative phosphorylation and protein synthesis (Liu *et al.* 2018). Thus, manipulation of single gene is not considered as the feasible choice. However, transcription factors involved in regulatory functions associated with lipid metabolism might be the significant tool to address the bottleneck of increasing the seed oil content (Wang *et al.* 2007; Zhang *et al.* 2016).

WRINKLED1 is a plant specific, ethylene-responsive transcription factor belonging to APETALA2 family containing two AP2 DNA-binding domains. The widely studied WRINKLED1 from *Arabidopsis thaliana* (ATWRI1; At3g54320) binds with the promoters of genes involved in glycolysis and fatty acid biosynthesis (Marchive *et al.* 2014; Liu *et al.* 2018). Two null alleles of *wri1* i.e., *wri1-3* (N585693) and *wri1-4* (N508559) were found in SALK collection (Alonso *et al.* 2003) which displayed T-DNA insertions in the 5th and 6th introns of *wri1* respectively. Transcriptome profile comparison showed the reduced expression of the genes involved in fatty acid and glycolytic enzymes in the *wri1-1* mutant seeds compared to wild type (Liu *et al.* 2018).

Seeds of loss-of-function mutant *Atwri1* display 80% reduction in triacyl glyceride (TAG) accumulation and increase in the sucrose level as compared to wild type, indicating that *Atwri1* is a regulator for allocating carbon

towards fatty acid or sucrose biosynthesis pathway. The splicing mutant showed a compromised glycolysis due to which the developing seeds were unable to convert sucrose into TAGs (Wu et al. 2014). Due to decreased fatty acid content these mutants show impaired seedling germination (Ma et al. 2015).

WRI1 affects seed oil production in plants of *Zea mays* (Pouvreau et al. 2011), *Brassica napus* (Liu et al. 2010; Wu et al. 2014) *Brachypodium distachyon* (Yang et al. 2015), *Brassica juncea* (Bhattacharya et al. 2016) and *Lepidium campestre* (Ivarson et al. 2017). Zang et al. (2018) have conducted genome wide identification of wrinkled1 genes from *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. raimondii* species and have confirmed that the ectopic expression of wri1a gene from *G. hirsutum* can rescue the seed phenotype in wri1-7 mutant of *A. thaliana*. *G. hirsutum* was transformed to produce *Ghwri1* over expressing lines and showed enhanced lipid content in seeds (Liu et al. 2018).

Some studies have shown that *WRINKLED1* gene from cotton is involved in cotton fiber development (Qu et al. 2012; Qaisar et al. 2017). The silencing of *wri1* in *G. hirsutum* enhanced fiber length and reduced oil content of seed, suggesting its positive correlation with fatty acid biosynthesis and negative with fiber elongation (Qu et al. 2012). All of the above studies were carried out with only *G. hirsutum wri1* genes. However, the transcriptional profile of *wri1* in elite fiber producing cotton (*G. barbadense*) and *G. hirsutum* exhibited marked differences (Qaisar et al. 2017). The expression of *wri1* was significantly higher in *G. barbadense* as compared to *G. hirsutum* and *G. arboreum* (Qaisar et al. 2017) indicating a positive correlation with fiber length. To date no direct information is available on involvement of any *wri1* of *G. barbadense* in fatty acid biosynthesis. Thus, a study was designed to investigate the possible role of GbWRI1 in seed development and biosynthesis of fatty acids in model plant *A. thaliana*. We transformed *Gbwri1* in loss of function mutant SALK_085693 of *A. thaliana* and performed morphological and molecular studies. As the transcription factor over-expression may result in some unwanted pleiotropic effects on certain morphological attributes in addition to expected increase of seed oil content, so its expression in model plant *A. thaliana* should be investigated. Therefore, the aim of the study was to clone the *Gbwri1* transcription factor gene from *G. barbadense* and to raise transgenic lines over-expressing this gene. The expected results might lead to the potential modulation of *GbWRI1* over expression directly in cotton in order to get crop with better fiber quality and increased oil contents.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted in research area of School of Biological Sciences, Punjab University, Lahore.

Arabidopsis thaliana wild type (Col-0) and wri1-3 mutant (SALK_085693) seeds were obtained from Arabidopsis Biological Resource Center, Ohio State University, OH, U.S.A. Seeds were surface sterilized with nascent Cl₂ produced as a result of reaction between commercial bleach and concentrated HCl and grown on half-strength MS medium (Sigma, St. Louis, Missouri, United States) containing 2% sucrose and 0.7% agar. Plates were incubated in darkness for 2 days at 4°C and were placed in a plant growth chamber at 17–20°C in a light/dark period of 16/8 h. After 10 days, the seedlings were transferred to soil in the growth chamber under the same conditions. Plants were watered regularly until flowers transformed to siliques. The whole plants were carefully bagged, dried and subjected to seed collection. The collected seeds were dried for further couple of days at 37°C.

Sequence retrieval, multiple sequence alignment and structure analysis

Nucleotide sequence of probe (GhiAffx.60562.1.S1_at) on Affymetrix cotton genome array (ThermoFisher Scientific) was retrieved from www.affymetrix.com as this probe showed involvement in fiber development (Qaisar et al. 2017). Full length sequence was retrieved by blasting the sequence of fiber related probe against *Gossypium barbadense* genome at cottonGen database (Yu et al. 2014). This sequence was named *G. barbadense wri1 (Gbwri1)* due to homology with *wrinkled1* transcription factor. Full length *Gbwri1* was isolated from *G. barbadense* var. Bar 14/5 cDNA as described (Qaisar et al. 2017) using gene-specific primers (Table 1) and was sequenced. *Atwri1* sequence was retrieved from The Arabidopsis Information Resource (TAIR). Homologues of *Gbwri1* in tetraploid cotton *G. barbadense* were identified through blastn using *Gbwri1* as query against the *G. barbadense* HAU v2.0 CDS (109,778) genome sequence database (Yu et al. 2014). The online translate tool (www.expasy.org) was used to predict the amino acid sequences of WRI1 homologues. ClustalX version 2.0 was used to perform multiple sequence alignments of all identified *wri1* homologues. Gene structures of all the *wri1* homologues in *G. barbadense* were generated using the Gene Structure Display Server. Homology model building of GbWRI1 proteins was done by uploading the amino acid sequence on SWISS-MODEL for structure prediction and homology building. 3-D structure of target protein was done based on sequence alignment between the target protein and the template structure.

Construct preparation and transformation in *A. thaliana*

RNA was extracted from *G. barbadense* fibers at 17 days post anthesis (DPA) as previously described (Qaisar et al. 2017). The cDNA was synthesized and amplified through polymerase chain reaction (PCR),

cloned into pTG19-T PCR cloning vector (Vivantis, Selangor Darul Ehsan, Malaysia) and confirmed by sequencing. Primers used for *Gbwri1* amplification were SF and WRIFCR1 (Table 1). After introducing the adaptors for recognition sites of restriction enzymes *NcoI* at 5' and *PmlI* at 3' termini, *Gbwri1* was cloned into pCambia 1301 plant binary vector between CAMV 35S promoter and NOS terminator. The recombinant plasmid (pSAM2) was electroporated into *Agrobacterium tumefaciens* strain LBA4404 and then transformed into *A. thaliana* by the floral dip method (Zhang *et al.* 2006). Transgenic plants were selected by growing them on MS medium containing hygromycin.

Molecular confirmation of transgenic lines

For the confirmation of integration of *Gbwri1* in transgenic lines, genomic DNA was extracted from *A. thaliana* leaf tissues of ten days old transformed lines and untransformed control plants using GeneAll® Exgene™ plant SV mini kit (Lisbon, Portugal) according to manufacturer's instructions. PCR was performed using sequence specific primers for *Gbwri1* SF and wriR520 (Table 1). PCR product was visualized using agarose gel electrophoresis.

Quantitative real-time PCR (qRT-PCR) analysis

Shoots of *A. thaliana* transformed plants were harvested at silique formation stage, ground in liquid nitrogen and total RNA was extracted using RNeasy Plant Mini kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Genomic DNA contamination was removed using DNase I (Qiagen, Hilden, Germany). First-strand cDNA synthesis was performed using Reverse Transcriptase (Promega, Madison, Wisconsin, United States). After optimization of gene specific primers (Table 1), relative expression of *wrinkled1*, biotin carboxyl carrier protein isoform 2 (*bccp2*) and keto-ACP synthase 1 (*Kas1*) was measured in transformants, wild type and wri1-3 mutant using real time RT-PCR as previously described (Qaisar *et al.* 2013, 2017). At least three biological replicates and three technical replicates for each genotype were used. *A. thaliana* actin gene (Table 1) served as endogenous control in these studies.

Determination of seed weight, size and scanning electron microscopy

Weight of hundred seeds from wild type (Col-0), wri1-3 mutant (SALK_085693) and transgenic *A. thaliana* lines was measured using a microbalance. The size of the seeds was determined by measuring the length and width of the seeds under a Light microscope (Optika Vision Lite Ponteranica, Italy) and using the software, Image J (<https://imagej.nih.gov/ij/>). The samples were mounted on carbon conductive tape pasted on aluminum sample holder and loaded into Scanning electron microscope (JOEL JSM-

6480LV, Freising, Germany) at 20kV for surface/morphology analysis.

Statistical analysis

One-way ANOVA and Bonferroni's multiple comparison test were performed in GraphPad Prism 5 software to evaluate the statistical significance of differences observed between wild type, wri1-3 mutants and transformed lines expressing *Gbwri1*.

Results

Gene structure and protein analysis of *Gbwri1* and its allelic isoforms in *G. barbadense*

G. barbadense is a tetraploid cotton (4X) harboring four alleles of *wrinkled1*. Alleles of *Gbwri1* in *G. barbadense* (*Gbar_D10G021070.1*, *Gbar_A10G020800.1*, *Gbar_D13G000170.1* and *Gbar_A13G000380.1*) were identified using blastn at cottonGen against *Gossypium barbadense* (AD2) genome HAU_v2 (www.cottongen.org). *Gbwri1* showed 99% amino acid sequence homology with *Gbar_D10G021070.1*. Gene models of *Gbwri1* and its four alleles were used to analyze the resemblance and diversity of their exon and intron structures (Fig. 1).

Gbwri1 and its homologues harbored a 5'UTR, 3'UTR, six exons and five introns. *Gbar_D10G021070.1* and *Gbar_A10G020800.1* showed close homology with *Gbwri1* while *Gbar_D13G000170.1* and *Gbar_A13G000380.1* were undersized at the C-terminal end of the gene. To investigate the amino acid sequence similarity and diversity among *Atwri1*, *Gbwri1*, and its homologues, multiple sequence alignment was performed. The comparison showed that *Gbwri1* was 10 amino acids shorter than *Atwri1*. While *Gbar_D10G021070.1*, *Gbar_A10G020800.1*, *Gbar_D13G000170.1* and *Gbar_A13G000380.1* were 2, 12, 58 and 68 amino acids shorter than *Atwri1*, respectively. Interestingly, the alignment showed that the two APETALA2 DNA binding domains were highly conserved in all WRI1 proteins (Fig. 2).

Maximum protein and nucleotide sequence similarity was observed between *Gbwri1* with *Gbar_D10G021070.1* indicating that *Gbwri1* is actually *Gbar_D13G000170.1*. Three dimensional structures based on homology modeling of the proteins of *Atwri1*, *Gbwri1* and its allelic forms were constructed (Fig. 3).

It was observed that DNA binding domains of *Gbwri1* were identical to the *A. thaliana* *wrinkled1* DNA binding domains in respect of their secondary structure. Both *Gbwri1* and *Atwri1* contain the same number of alpha helix and beta sheets. However, the helices had more turns and beta sheets were smaller in cotton isoforms in comparison with *Atwri1* (Fig. 3). APETALA2 DNA binding domains which bind to the DNA through tryptophan and arginine residues in the beta sheets were conserved in beta sheets of both AtWRI1 and GbWRI1 domains.

Table 1: Sequences and purpose of the primers used in this study

Primer Pair	5'-3' sequence	Product (bp)	Accession number	Objective
SF	GATCAATCAGAGAAACCCAAGG	1300	<i>Gbar_D10G021070</i>	<i>wrinkled1</i> full gene amplification
WRIFCR1	TTAAACAGAGTAGTTACAAGAAACC			
SF	GATCAATCAGAGAAACCCAAGG	351	<i>Gbar_D10G021070</i>	<i>Gbwri1</i> confirmation in transgenic lines
wriR520	TGTCACITTTCTTCATTTCTCCATCTCTTT			
Arabi actin F	TGATTGGTATGGAGAATGCTGGTAT	159	AT2G37620	Housekeeping gene for normalization of gene expression.
Arabi actin R	CAGTGATCTCTTTTGCTCATCTGTC			
<i>kas1F</i>	CTTCTGCGCTTCAAACCCCTGA	204	AT5G46290	Expression analysis of <i>kas1</i>
<i>kas1R</i>	TTCCGCAGACAAGAGAGAGAAC			
<i>bccp2F</i>	GGTTTCGGATTTCTGCTTGTCG	132	AT5G15530	Expression analysis of <i>bccp2</i>
<i>bccp2R</i>	GACAGAGAGCCAGGATTGAGAG			

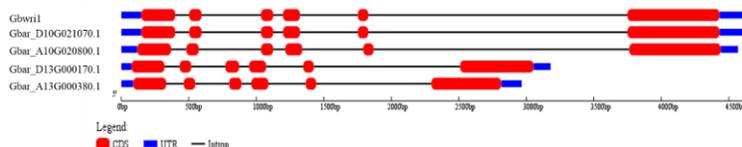


Fig. 1: Gene structures of *Gbwri1* and its homologues in tetraploid cotton *G. barbadense* showing distribution of intronic and exonic regions

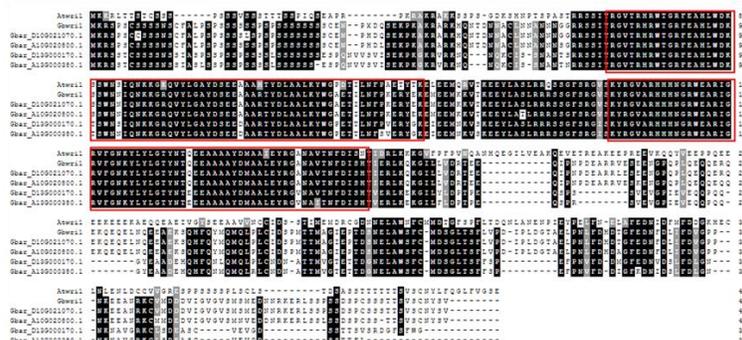


Fig. 2: Protein sequence alignment of *Atwri1*, *Gbwri1* and *wri1* homologues in *G. barbadense*. Identical residues are shaded black, similar residues are shaded gray while AP2 domains are indicated by red boxes

A. thaliana mutant and gene complementation

Homology of *Atwri1* and *Gbwri1* in gene structure (Fig. 1 and 4), amino acid sequence (Fig. 2), 3D structure (Fig. 3) and DNA binding domains suggests functional homology between two homologues. Although *Gbwri1* is involved in fiber development and *Atwri1* plays an important role in seed filling and fatty acid biosynthesis. In order to test whether *Gbwri1* can complement *Atwri1*, a null allele of *wri1* namely, *wri1-3* (N585693) was identified in the SALK collection displaying T-DNA insertions in the 5th intron of *wri1* (Fig. 4).

We cloned *Gbwri1* in pCambia1301 plant expression vector between cauliflower mosaic virus (CaMV) 35S promoter and nopaline synthase (NOS) terminator (Fig. 4A) and transformed in *wri1-3* mutant of *A. thaliana* using *Agrobacterium* mediated transformation.

Integration and expression of *Gbwri1* in *A. thaliana*

To confirm the integration of *Gbwri1* in transgenic lines of

A. thaliana, PCR was carried out with genomic DNA templates using gene-specific primers for *Gbwri1* sequence. Agarose gel electrophoresis confirmed the presence of 351bp product in A1, A3 and A5 transgenic lines of *A. thaliana* harboring *Gbwri1* cDNA (Fig. 5A). *G. barbadense* cDNA was used as a positive control. To check the transcription of *Gbwri1* in the transgenic lines, mRNA levels were checked in the transformed and untransformed lines of *A. thaliana* using real-time RT-PCR. We found that A1 and A3 showed significantly higher transcript levels (6- and 9-fold respectively) of *Gbwri1* as compared to untransformed mutant (Fig. 5B).

Gbwri1* resumed the expression of fatty acid biosynthesis genes and seed weight in *wri1-3* mutant of *A. thaliana

We checked the expression of *bccp2* (AT5G15530) and *kas1* (AT5G46290) in the mutant and transgenic lines in order to see whether *Gbwri1* has resumed the function of *Atwri1* by enhancing the expression of these target genes. Transcript accumulation was dropped 15-fold in the mutant

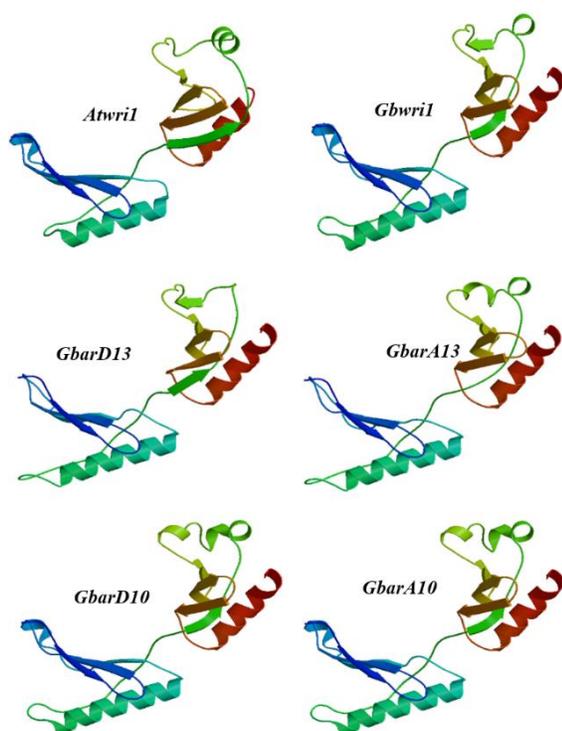


Fig. 3: Three-dimensional protein structures of *Atwri1*, *Gbwri1* and *wri1* homologues in *G. barbadense*.

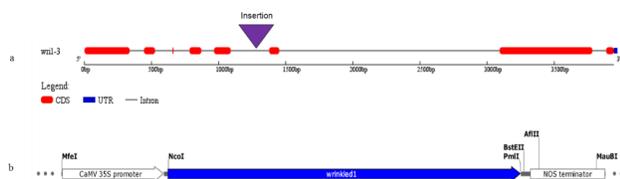


Fig. 4: Complementation of *wri1-3* mutant of *A. thaliana* with *Gbwri1* expression cassette. (A) Gene model of *A. thaliana wri1-3* mutant showing the site of T-DNA insertion. (B) T-DNA element of pSAM2 plasmid harboring *Gbwri1* coding region from *G. barbadense*

as compared to wild type while transformation with *Gbwri1* in A3 line partially resumed the expression to a level half than the wild type (Fig. 6A). While in A1 line, the expression increased non-significantly as compared to *wri1-3* mutant. Transposon insertion in *wri1-3* mutant reduced the expression of *kas1* gene 8.5 fold as compared to wild type. This reduction in expression was compensated by transformation of functional *Gbwri1*, up to 3 fold in A1 and 4 fold in A3 line in comparison with mutant (Fig. 6B).

Ectopic expression of *Gbwri1* rescued the seed morphology of *wri1-3* mutant of *A. thaliana*

Seeds of T3 homozygous progeny of transgenic lines were investigated and compared with wild type and *wri1-3* mutant. Microscopic observation of the mature seeds

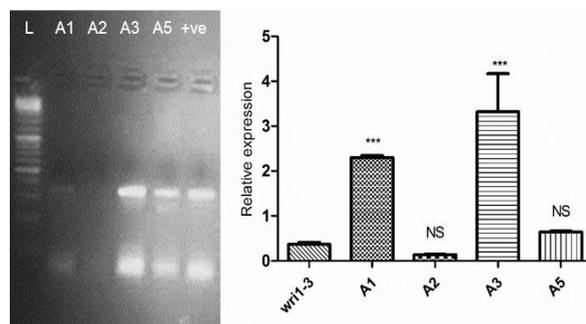


Fig. 5: Confirmation of transgene in transgenic lines of *A. thaliana*. (A) Amplification of *Gbwri1* in DNA isolated from transgenic lines of *A. thaliana*. (B) Transcript levels of transgene in transformed lines. At least three biological replicates of each genotype were used. Error bars represent standard deviation, ns indicates P -value is greater than 0.05. *** means P -value is less than 0.001

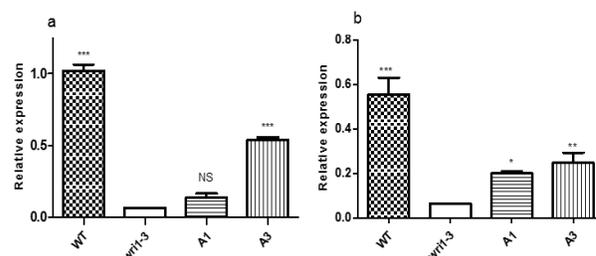


Fig. 6: Transcription of WRI1 target genes in transgenic and parent lines (A) *bccp2* gene (B) *Kas1* gene. At least three biological replicates of each line were used for expression analysis. NS, $P > 0.05$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$

showed that *wri1-3* mutant seeds were much smaller in size as compared to wild type while a reversion of wrinkled phenotype was observed in transgenic lines (Fig. 7A).

The seed surface analysis indicated that A3 line showed complete reversion while A1, A5 and A2 exhibited partial reversion (Fig. 7B). In addition to seed surface, further seed traits were observed in transgenic lines. Examination of seed weight (per 100 seeds) showed that ectopic expression of *Gbwri1* significantly increased the seed weight in transgenic lines (Fig. 4B). We observed that the seed weight was increased by 30.9%, 24.2%, 19.3% and 19.3% in transgenic lines (A1, A2, A3 and A5) respectively as compared to mutant (Fig. 8A). In agreement with increased seed weight, seed area was also significantly increased as compared to wild type. The *wri1-3* mutant showed a 26 percent reduction in seed area as compared to wild type seeds which was resumed in transgenic lines to the wild type (Fig. 8B).

Impaired germination and seed phenotype of *wri1-3* mutant is rescued by *Gbwri1*

During early plant growth, TAG reserves are broken down

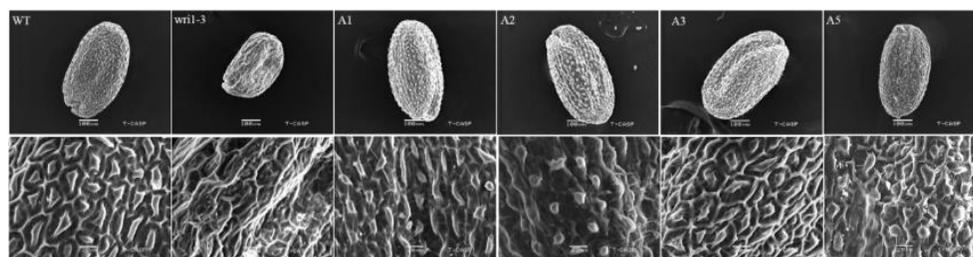


Fig. 7: Comparison of wildtype (WT), mutant (*wri1-3*) and transgenic lines (A1, A2, A3 and A5) of *Arabidopsis thaliana*. (A) Mature seeds. (B) Scanning electron microscopic images of seed surfaces

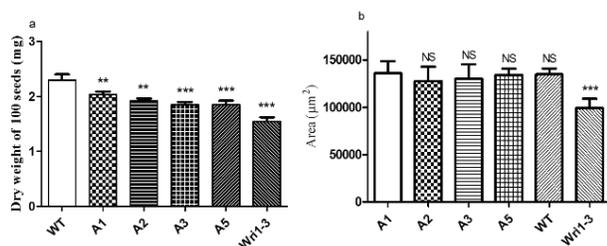


Fig. 8: Ectopic expression of *Gbwri1* rescues seed of *Arabidopsis thaliana*. A.100 Seed dry weight and B, seed area. Length of the bar represents the mean value of triplicates. Error bars depict standard deviation. *** indicates P value < 0.001 , ** P value < 0.01 and NS represents P value > 0.05 .



Fig. 9: Growth of wild type, mutant and transgenic lines (A1 and A3) of *Arabidopsis thaliana* on sucrose free medium. Error bars represent standard deviation

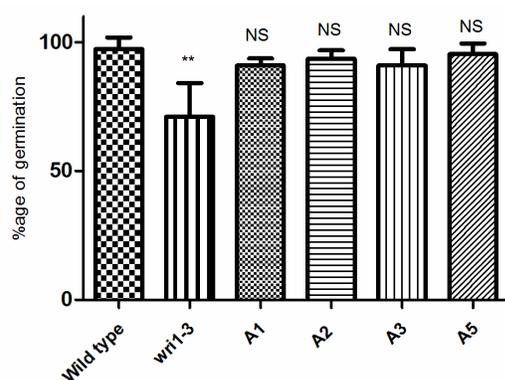


Fig. 10: Seed germination rate of wild type, *wri1-3* mutant, and transgenic lines of *Arabidopsis thaliana*. NS represents $P > 0.05$; ** $P < 0.01$

into soluble sugar molecules which can be transported to the developing seedlings (Pritchard *et al.* 2002). The *wrinkled1*

mutants of *A. thaliana* had reduced seed storage compounds required for normal seed development (Ma *et al.* 2013). Sucrose in the medium is required as building blocks and energy supply during *wri1-3* seedling establishment. Due to decreased fatty acid content of *wri1-3* mutants the seedling germination and establishment were impaired in the medium without sucrose. The *wri1-3* mutant displays reduced seed germination and root development on sucrose-free medium (Fig. 9).

However, the transformation of *Gbwri1* resumed seed germination and root growth to the wild type. Transgenic *wri1-3* mutants expressing *Gbwri1* developed normal seedlings on the medium lacking sucrose (Fig. 9). To further confirm the reduced seed germination in *wri1-3* mutant, the plants were grown on MS agar medium. The *wri1-3* mutants showed a 27% reduced seed germination compared to the wild type, while the germination of A1, A2, A3, and A5 transgenic lines was equivalent to the wild type (Fig. 10).

Discussion

WRINKLED1 protein is an APETAL2 like transcription factor that binds with the promoters of fatty acid biosynthesis and carbohydrate metabolism genes and is involved in regulation of oil accumulation during maturation of *A. thaliana* seeds (Junker *et al.* 2012). A *WR11*-like gene from *B. napus* (*BnWR11*) showed 80% sequence identity with *AtWR11* and overexpression of which increased seed mass and oil content in transgenic *A. thaliana* (Liu *et al.* 2010; Kong *et al.* 2020). This loss of function mutant line produces wrinkled seeds and the transcript levels are severely reduced as compared to wild type (Junker *et al.* 2012). Like *Atwri1*, the seed-expressed *Ghwri1* orthologs have been observed regulating seed oil biosynthesis by not only complementing the *wri1-7* but also by increasing the seed oil content (Liu *et al.* 2018; Zang *et al.* 2019; Correaa *et al.* 2020). The involvement is further confirmed by the silencing of *G. hirsutum wrinkled1* gene which resulted in enhanced cotton fiber length and reduced fatty acid biosynthesis by partitioning the carbon flow (Qu *et al.* 2012). We have previously reported that *G. barbadense*, which possesses marked differences in cotton

fiber morphology, revealed elevated expression of *Gbwri1* transcription factor compared to *G. hirsutum* (Qaisar *et al.* 2017). Moreover, *Gbwri1* expression in *G. barbadense* is positively correlated with fiber length in contrast with *G. hirsutum WRINKLED1*. This unusual behavior led us to further investigate the possible role of *Gbwri1* seed development and biosynthesis of fatty acids in model plant *A. thaliana*.

In this study we inquired whether GbWRI1 which is more highly associated with higher fiber length might have evolved modified traits associated with this function. It was further investigated whether GbWRI1 could function in seed oil synthesis regulation or result in modified phenotypes regarding germination and seedling development. GbWRI1 can complement germination and seedling development phenotypes of *wri1* in *A. thaliana* have not been studied previously. This is the first report highlighting the role of *WRI1* allele of *G. barbadense* in *A. thaliana*.

WRI1 transcription factor induced fatty acid buildup leading to oil accumulation in seeds of *A. thaliana* and mutation of AtWRI1 failed to produce seed oil resulting in deformed seeds with wrinkled appearance, less surface area and dry weight (Junker *et al.* 2012; Wu *et al.* 2014). In *wri1-3* loss of function mutant, expression of target genes of *Atwri1*, *i.e.* *bccp2* and *kas1* is reduced leading to decreased fatty acid content, Where we report that ectopic expression of GbWRI1 in *A. thaliana* mutant (*wri1-3*) lead to overexpression of oil accumulating genes *bccp-2* and *kas-1* which effected seed weight, size, germination rate and seed morphology. The similar findings were observed in *G. hirsutum* where overexpression of *Ghwri1* enhanced lipid content in seeds (Liu *et al.* 2018; Correea *et al.* 2020). Like GhWRI1, GbWRI1 shows similarity in gene structure (Fig. 1 and 4), amino acid sequence (Fig. 2), 3D structure (Fig. 3) and DNA binding domains with AtWRI1. These results indicate that due to functional homology between two homologues, this GbWRI1 can rescue the *wri1-3* mutant seed phenotype probably by regulating fatty acid biosynthesis.

Our study also revealed that transformation of GbWRI1 rescued the seed germination and root growth in *wri1-3* mutants. During early plant growth, TAG reserves are broken down into soluble sugar molecules which can be transported to the developing seedlings (Pritchard *et al.* 2002). The *wrinkled1* mutants of *A. thaliana* had reduced seed storage compounds required for normal seed development (Ma *et al.* 2013). Sucrose in the medium is required as building blocks and energy supply during *wri1-3* seedling establishment. Due to decreased fatty acid content of *wri1-3* mutants the seedling germination and establishment were impaired in the medium without sucrose. GbWRI1 also regulates glucogenesis in the absence of sucrose, leading to seedling establishment (Fig. 9) which emphasizes on the role of *WRI1* in sugar metabolisms. As cellulose constitutes 85–97% of dry weight of cotton fiber

and sugar molecules serve as precursors for cellulose biosynthesis (Pattathil *et al.* 2015; Cosgrove 2018; Herger *et al.* 2019; Vaahtera *et al.* 2019; Rui and Dinneney 2020) so GbWRI1 regulates fiber development through regulating sugar metabolism. Moreover, GbWRI1 shows higher expression at later stages of fiber development (secondary cell wall biosynthesis) which predominantly are involved in cellulose deposition.

Conclusion

The novel *Gbwri1* gene has been observed to be involved in the regulation of seed lipid content in *A. thaliana*. Overexpression of *Gbwri1* in native cotton varieties can be studied with reference to cotton fiber improvement and enhancement of seed oil content. This gene may be the potential candidate to provide an opportunity of commercial use for breeding transgenic crops.

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Author Contributions

MI performed experiments; SB performed experiments; AM data analysis; SY data analysis; TR bioinformatics; MB paper write-up; TH Scanning Electron Microscopy; Uzma Qaisar acquired funding, experiment design and performed manuscript writing

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