Allelic Variations in 5' UTR of TaAFP-B Effecting the Seed Dormancy and Other Agronomic Traits in Transgenic Wheat

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Allelic variations in 5' UTR of *TaAFP-B* effecting the seed dormancy and other agronomic traits in transgenic wheat

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ABSTRACT:

Background:

TaAFP (*Triticum aestivum* L. ABA insensitive five binding protein) is the homology of *AFP* of *Arabidopsis thaliana* which was a negative regulator in ABA signaling and regulated embryo germination and seed dormancy. *TaABI5* (*Triticum aestivum* L. ABA insensitive five) gene was seed-specific, and accumulated during wheat grain maturation and dormancy acquisition, which played an important role in seed dormancy. In our previous study, two allelic variants of *TaAFP* were identified on chromosome 2BS in common wheat, and designated as *TaAFP-B1a* and *TaAFP-B1b*. Sequence analysis showed a 4-bp insertion in the 5’ UTR region of *TaAFP-B1a* compared with *TaAFP-B1b*, which affected the mRNA transcription level, mRNA decay, translation levels of GUS and tdTomatoER, GUS activity, and was significantly associated with seed dormancy in common wheat.

Results:

The results of transgenic wheats showed that: the genotypes of average GI values, plant height, grain weight of hundred and rough of second and third stem node are all significantly more in pUbi-TaAFP-BaS transformed wheat plants than in pUbi-TaAFP-BbS transformed ones, but transcript expression level.

Conclusion: Above all dates indicated that the 4-bp insertion in the 5'UTR of *TaAFP-B* decreased the transcript expression level of *TaAFP-B* and the PHS resistance, and increased the plant height, grain weight of hundred and lodging

resistance in this system of over expression transgenic wheat.

Key words: *TaAFP-B*, 5'UTR, Allelic variation, Transgenic wheat, Agrobacterium-mediated Transformation

INTRODUCTION

Pre-harvest (PHS) sprouting is a worldwide severe problem that leads to loss of grain weight and reduction in the end-use quality of kernels in wheat (Groop et al. 2002; Humphreys et al. 2002). Severely sprouted wheat becomes suitable only for animal feed and cannot be used for flour production or any other application in the food industry (Simsek et al. 2014). Therefore, understanding the genetic mechanism(s) of seed dormancy in wheat became more and more important.

The mechanism of ABA sensitivity in seeds has been extensively studied in *Arabidopsis*. Some genes associated with seed dormancy have been identified as factors in the ABA signaling and ABA synthesis pathway (Jiang et al. 2003; Ye et al. 2016; Zhang et al. 2010; Hindson et al. 2011). *ABI3*, *ABI4*, and *ABI5* are positive regulatory factors, which roles of *ABI3*, *ABI4*, and *ABI5* also was affecting seed development and ABA sensitivity, but null mutation of *abi3* is more severe than that of *abi4* or *abi5* (Parcy et al. 1994; Finkelstein et al. 1998; Finkelstein et al. 2000). Negative regulators are *ABI1*, *ABI2*, and AIP2 E3 ligase for *ABI3* and RING E3 ligase and ABA insensitive five binding protein (AFP) for ABI5 (Gosti et al. 1999; Lopez-Molina et al. 2003; Zhang et al. 2005; Stone et al. 2006). *ABI3* and *ABI5* act as intermediates, regulating the maturation and germination of seeds and expression of *ABI3* and *ABI5* facilitates desiccation tolerance of the seed in later stages of maturity (Lopez-Molina et al. 2001; 2002; Carles et al. 2002). The *Arabidopsis ABI3* gene which is required for appropriate *ABI5* expression (Cheng et al. 1997; Vaucheret et al. 1998; Finkelstein et al. 2010), encodes a transcription factor and acts together with *ABI5* to direct embryonic gene expression and seed sensitivity to ABA (Ray et al. 2017; Lopez-Molina et al. 2003; Finkelstein et al. 2003). *ABI5* acts as a critical factor in maturation, dormancy development of seeds, or the dehydration tolerance of young seedlings of *Arabidopsis* (Jiang et al. 2003; Jones et al. 2005).

ABI5 binding protein (AFP) was isolated by yeast two-hybrid assays, which played a negative regulator of ABA signaling pathway by facilitating the degradation of ABI5.

The transcription and translation of *AFP* gene increased during seed development and desiccation, ultimately reaching plateau values in mature seeds (Cheng et al. 1997). Three homology *TaAFPs* in wheat were isolated, *TaAFP-A*, *TaAFP-B* and *TaAFP-D*, located on the short arms of chromosomes 2A, 2B and 2D, respectively (Naruhito et al. 2008). In our previous study, two allelic variants of *TaAFP-B* were identified and designated as *TaAFP-Ba* and *TaAFP-Bb*，compared with *TaAFP-Ba*, a 4-bp deletion in the 5'UTR region was detected in *TaAFP-Bb*, and this 4-bp deletion structure affected the mRNA stability, mRNA transcription expression level, translation expression level of tdTomatoER and GUS, and GUS activity. and the 4-bp insertion in the 5'UTR of *TaAFP-B* increase the GUS activity in experiment of GUS activity of P*TaAFP-Ba* (with 4-bp insertion)::GUS transgenic rice, decreased the transcript expression and translation level of *TaAFP-B* in transgenic rice. So, *TaAFP-B* was significantly associated with PHS tolerance in common wheat (Feng et al. 2019).

Therefore, in order to further clear the function of the differential structure in the 5'UTR of *TaAFP-Ba/b*, and contribution of the mechanisms underlying seed dormancy or PHS tolerance in wheat, system of transgenic wheat would established to study the affect of the differential sequence in 5'UTR of *TaAFP-B* on promotor activity, transcription and phenotypes of over expression of *TaAFP-Ba/b* in transgenic wheat.

MATERIALS AND METHODS

Plasmid Constructions

pCAMBIA1390-Ubi-GFP vector were kindly supplied by the professor Xia Lanqing (Chinese Academy of Agricultural Sciences, CAAS)). Then, Target genes fragment of *TaAFP-Ba/bS* manipulation was performed and cloned into the vector by General Biosystems (Anhui) Co. Ltd. Respectively, we successfully constructed the recombination expression vector of Ubi-*TaAFP-Ba/bS* to determine the function of 5'UTR of the two allelic *TaAFP-Ba* and *TaAFP-Bb* in regulation mechanism of resistant to pre-harvest sprouting.

Transformation of Transgenic Wheat

The recombination plasmids as described previously (Duan et al. 2012). Moreover, Ubi- *TaAFP-Ba/bS* were transformed to the embryonic calli of wheat line K35, which operated by Shandong Academy of Agricultural Sciences. The transformed calli were screened and planted to regeneration under the selection pressure of hygromycin, and seeds of T1 were acquired.

All of plant material used in this study were cultured in National Transgenic Safety Centre (36°42'28''N, 117°05'19''E), identified by authors Yumei Feng and Yang Han, and stored in Key Lab of Germplasm Innovation and Utlization of Triticeae Crop at Universities of Inner Mongolia Autonomous Region. And a voucher specimen of these material has not been yet deposited in a publicly available herbarium.

Identification of Transgenic Positive Plants and Copy Number

All of the lines seedling in transgenic wheat and 25 lines for each transgenic type in transgenic wheat were cultivated discretionary in . And then, genomics DNA was isolated from the young leaves of mixed plants of everyone lines (T1) according to the

method of DNA secure Plant Kit bought TIANGEN Biotech. PCR reaction were performed as described previously to identify the transgenic positive lines (Feng et al. 2017). Amplified PCR fragments and primer were showed in Table 1. After then, quality of genomic DNA of transgenic positive lines measured by BioDrop spectrophotometer, and copy numbers were detected the using method of ddPCR described protocol of Ray Collier et.al by Shanghai Biotechnology Co. Ltd. A pair of primers and probes designed to detect a unique single-copy for target and endogenous reference gene, respectively, the targets and endogenous reference were *DD-TaAFP-B* and *TaPINb-D1b*^{d} in wheat (Table 2). The reference gene amplicons were detected using FAM™-labeled probes, while the target genes amplification were detected with VIC™-labeled probes. The ddPCR reaction mixture consisted of 2ul DNA, 1X QX200 EvaGreen Supermix 10ul (Bio-Rad), 450nM of each primer pair and probe 250nM of each probe. Thermal cycling conditions were 95°C for 10 min, followed by 40 cycles of 94°C for 30s, 60°C for 30s, with 98°C for 10min and a final hold at 4°C. The detail experimental procedures were referenced from Per H (Per et al. 2016) and Ray Collier (Ray et al. 2017). Lastly, we planted and identified the transgenic positive individual plants at least three independent single-copy lines for each transgenic type under 25°C in the National Safety Experimental Base of GMO, Jinan City, Shandong province of transgenic wheat to further analysis.

Table 1 Primers used in this study

Primer Set	Upstream (5'-3')	Downstream (5'-3')	Primer Anneal Temperature (℃)	Fragment Size (bp)

UB F/R	TTTGTTCGCTTGGTTGTGA	AGCTCGACCACCTCGTCG	58	613
Taactin F/R	GTTTCCTGGAATTGCTGATCGCAT	CATTATTTCATACAGCAGGCAAGC	62	410
Q-TaAFP-B F/R	ACCTCCTCAAGCATGCCGG	ACTTGTTCTGGTTGCTGGCA	66	102
Q-TaAFP-A F/R	CCCTCCTCAAGCATGCCCG	GCTGGCATTGTTATTATTATCG	59	100
Q-TaAFP-D F/R	GCTTCCTCAAGCATGCCGGA	CGTCCACCTTGGAGGAGACT	56	289
Q- TaABI5 F/R	GGAAGAAGTCACCTCRCACC	GAGGCAAGGAGAACGACT	62	310

Observation and analysis of transgenic plants for phenotype

The single copy positive individual plant of each lines were selected to analyse the phenotypes after RT-qPCR avoiding the affection of copy number in transgenic wheat. The T2 generations were observed and analysed for height, tillering, stem length, and the germination index, et. al until the plants reached the wax ripening stage.

Expression Analysis

Total RNA was extracted from 5g tissues with the TaKaRa MiniBEST Plant RNA Extraction Kit. The cDNA was synthesized and RT-qPCR reactions were tested by

LightCycler®480 System Real Time PCR as the described from Y.M Feng et al. 2019. All primers of the target gene were listed as in table 1. Relative expression of target genes were evaluated by the 2^-ΔΔCt method (Li et al. 2018). The average was calculated from three biological replicates, and the error bars represent ‘‘± standard deviation’’ (± SD).

Germination assays and Stress treatments

The seeds of T2 lines with single-copy were sterilized in ethyl alcohol of 75% to ensure that all of the fresh seeds were similar maturity and the growth chamber under normal conditions with 16/8 h light/dark cycles at 25°C in wheat. For germination assays, the seeds of 100 were germinated for 7 days, the GI values of each construction were calculated according to the number of germinated seeds every day. For the stress treatment, the seeds were incubated with solid 0.5x MS medium plus 3uM ABA, 150 mM NaCl (for salt stress) and 300 mM Mannitol (for dehydration stress), respectively, at the same conditions for 1, 2, 3, 4 and 5 days. Transgenic seeds of non-recombination were used as control and every one experiment has three biological replicates. All samples were collected after each treatment in everyday, frozen in liquid nitrogen, and stored at -80°C condition.

RESULTS

Identification of Positive Plants and Analysis of the Copy Number

Total 25 lines of *TaAFP-Ba/bS* transgenic wheat (T1) were selected under the

selection pressure of hygromycin. Then, using the specific primer set UBF/R, the frequency of positive lines of *TaAFP-Ba/bS* transgenic wheat was 76% in transgenic wheat (Table 1).

The copy numbers of transgenic positive lines were detected by method of droplet digital PCR (ddPCR) (Table 2 and Fig. 1). Further analysis showed that the frequency of single copy of *TaAFP-Ba/bS* was 46.67% in transgenic wheat (Table 3). Then, in order for identification of phenotypes of these copy number transgenic plants, these single-copy lines of transgenic wheat were planted, and further identified the positive individual plant using special primers set (Table 1). The results showed that the frequency of *TaAFP-Ba/bS* positive individual plants were 36.6% in transgenic wheat.

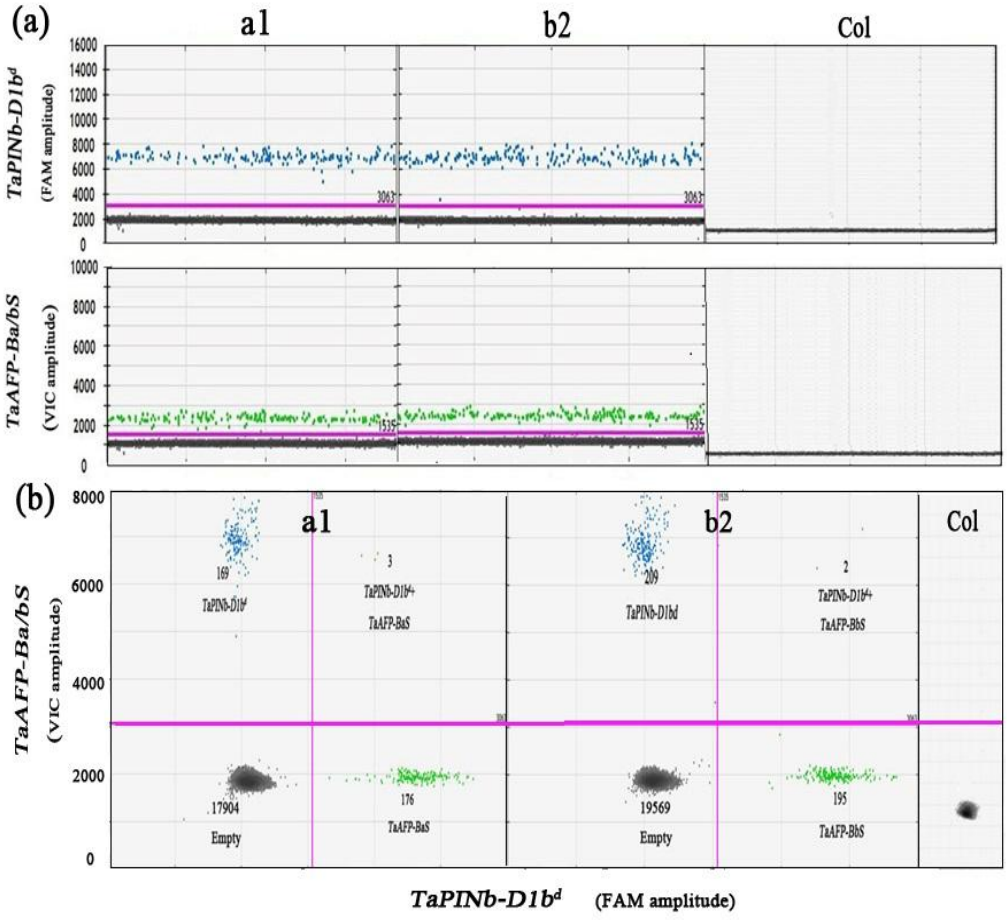

Fig. 1 Droplet digital polymerase chain reaction (ddPCR) data output from QuantaSoft™

Application illustrating measurements taken for the *TaAFP-Ba/bS* one copy number from transgenic wheat lines. **a1:** transgenic wheat line by transforming *TaAFP-BaS* gene; **b2:**transgenic wheat line by transforming *TaAFP-BbS* gene; **Col:** transgenic wheat by transforming non-recombination plasmid pCAMBIA1390. **(a)** One-dimensional plot of droplets measured for fluorescence signal emitted from the endogenous reference gene *TaPINb-D1b*^{d}(FAM™ labeled; positive droplets are blue) or the transgenic *TaAFP-Ba/bS* (VIC™ labeled; positive droplets are green). Negative droplets are shown in black. **(b)** The droplets visualized in two dimensions for line a1, b2 and col. The colors are as described in (a), except that droplets containing both fluorescent probes are orange.

Table 2 The primer and probe sequences used in ddPCR

Transgene	Primer sequences (5'-3')	Probe sequences (5'-3')	Temp (℃)	Amplicon (bp)
TaPINb-D1b^{d}	AGTTGGCGGCTGGTACAATG **(F)**	**FAM**-TCAACAATGTC		
	ACATCGCTCCATCACGTAATCC **(R)**	CGCAGGAGCG-**BHQ-X**	60	106
DD-TaAFP-B	TGCTCACCCTGTTGTTTGG **(F)**	**VIC**-ACTAGGTACCCTTCC		
	AAGCAGACGCCAATCCCT **(R)**	**-BHQ-X**	57	99

Expression of the *TaAFP-B* gene in single-copy transgenic plants

Because the copy numbers was related to the expression quantity, so single-copy *TaAFP-Ba/bS* transgenic wheat were selected, and transcript expression levels of *TaAFP-B* in seeds was measured by RT-qPCR, and non-recombination vector was

transformed in wheat as control. The higher transcript expression levels of *TaAFP-BaS* were observed, the more copy number ratios in transgenic wheat have existed. Moreover, there was significantly different among the different copy numbers of *TaAFP-Ba/bS* in transgenic wheat (Table 3). So three lines closing to one copy number of *TaAFP-Ba/b* in transgenic wheat were selected to measured the transcript expression level. The results showed that the transcript expression levels of *TaAFP-BaS* were lower than that of *TaAFP-BbS* in transgenic wheat mature seeds (Fig.2).

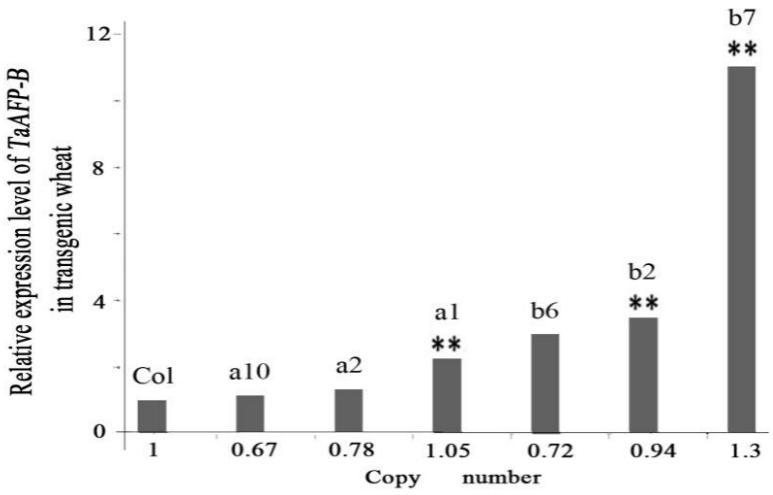

Fig. 2 The expression levels of *TaAFP-B* in transgenic wheat mature seeds with single-copy of *TaAFP-Ba/bS*

Table 3 The copy number and ratio of the genetically modified wheat ***TaAFP-Ba/bS*/*TaPINb-D1b*^{d}** gene by **ddPCR**

sample	*TaPINb-D1b*^{d}	*TaAFP-Ba/bS*	Copy	sample	*TaPINb-D1b*^{d}	*TaAFP-Ba/bS*	Copy

	（copies/ul）	(copies/ul)	numbers of target gene		（copies/ul）	(copies/ul)	numbers of target gene
a1	1110	1160	1（1.05）	b4	1890	79	0（0.04）
a2	1720	1350	1（0.78）	b6	1620	1170	1（0.72）
a3	1890	310	0（0.16）	b7	1750	2270	1（1.30）
a4	1660	15900	10（9.58）	b9	700	1330	2（1.9）
a10	1230	820	1（0.67）	b11	1260	560	0（0.44）
a11	1100	460	0（0.42）	b12	890	510	1（0.57）
b2	1250	1170	1（0.936）	b13	1370	10600	8（7.74）
b3	2080	5410	3（2.6）	Col	1250	1072	1（0.86）

In addition, the expression pattern and level of transgenic wheat in different tissues (root, stem, leaves) of 30 DAG and mature seeds of T2 were detected by RT-qPCR with Q-TaAFP-B-F/R primers (Table 1). The expression level tendency of *TaAFP-B* was seeds > roots > stems > leaves and significantly increased in transgenic plants compared with the control (Fig. 3).

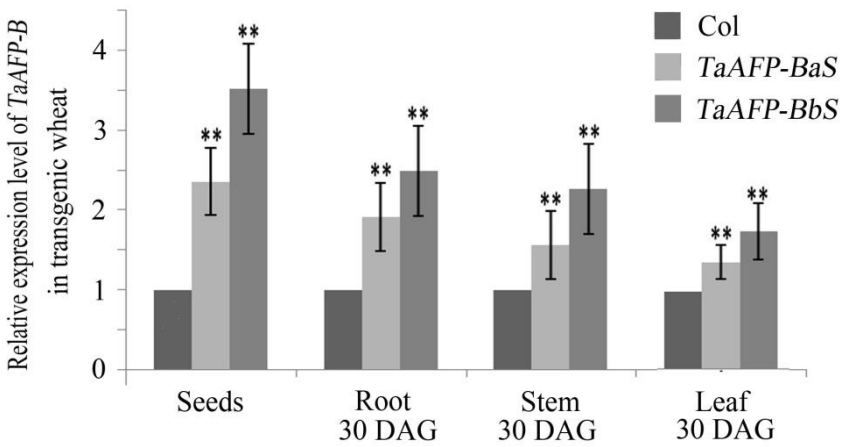

Fig. 3 The expression levels of *TaAFP-B* in different tissues of transgenic wheat with single-copy of *TaAFP-Ba/bS*

In order to clear level and change of transcript expression level of *TaAFP-B*, *TaAFP-A*, *TaAFP-D* and *TaABI5* in single-copy transgenic wheat, further experiment of RT-qPCR was selected, the results showed that 1) the expression of *TaAFP-B* gene transcript was always higher than *TaAFP-A* and *TaAFP-D* in seeds, which was consistent with the expression trend of common wheat; 2) the transcript expression levels of *TaAFP-BaS* are always lower than *TaAFP-BbS* in transgenic wheat, which was contrary to the results of common wheat; 3) there were significant differences in the expression levels of *TaAFP-BaS* and *TaAFP-BbS* in the seeds of single copy wheat lines, and which both have higher levels than the control; 4) the expression levels of *TaABI5* was not significantly different between the *TaAFP-BaS* and *TaAFP-BbS* transgenic materials, indicating that the overexpression of *TaAFP-BaS* and *TaAFP-BbS* did not cause the significantly change of *TaABI5* transcript expression (Fig. 4).

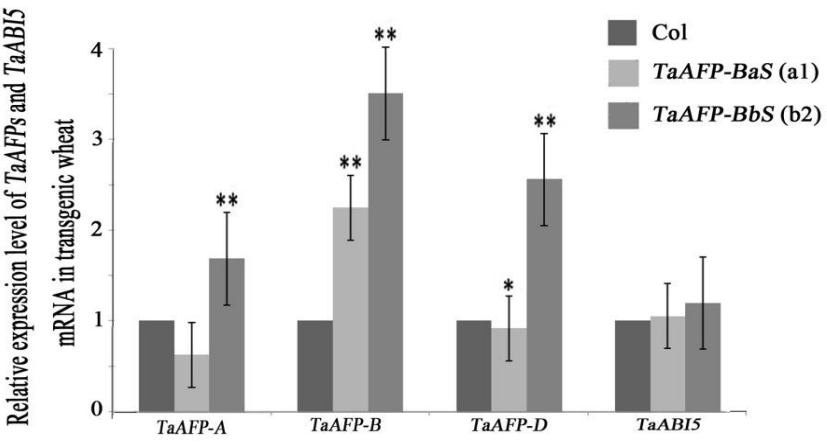

Fig. 4 The expression levels of *TaAFPs* and *TaABI5* located on A, B, and D chromosome, respectively, in transgenic wheat with single-copy of *TaAFP-Ba/bS*

The transcript expression of *TaAFP-B* induced by abiotic stress

In order to clear reflection of transgenic wheat under the abiotic stress respons e, further transcript expression pattern and level of *TaAFP-B* in *TaAFP-Ba/bS* with different stress were detected, and seeds of T2 were treated by ABA (3 mM), Salt (150 mM, NaCl) and dehydration (300 mM, Mannitol) for 1 to 5 days, respectively. In all treated material, the transcript expression levels of *Ta AFP-B* in *TaAFP-BaS* transgenic wheat always was lower than *TaAFP-BbS*, an d there both had signifcantly higher level in *TaAFP-BaS* and *TaAFP-BbS* trans genic wheat, respectively, than in contral (Fig. 5). In addition, there had more obvious change of transcript expression levels of *TaAFP-B* in *TaAFP-BaS* and *TaAFP-BbS* seeds treated by ABA and NaCl than treated by Mannitol, which demonstrated that transgenic wheat of *TaAFP-BbS* and *TaAFP-BaS* were more sensitive to ABA and NaCl than Mannitol; compared with control treated by w ater for 1d, 2d, 3d and 4d, respectively, the transcript expression levels decreas ed in *TaAFP-BaS* transgenic wheat and increased in *TaAFP-BbS* treated by AB A, NaCl and Mannitol (Fig. 5). These dates indicated that different sensitive to ABA and NaCl than Mannitol were existed in transcript expression level of tr ansgenic wheat system of *TaAFP-BbS* and *TaAFP-BaS.*

The differences and correlation of the expression level between *TaAFP-B* an d *TaABI5* under different stress treatments were detected in transgenic wheat, t he dates showed that 1) the expression level of *TaABI5* in *TaAFP-BaS* was lo wer than in *TaAFP-BbS*; 2) among them, highest transcript expression level of *TaAFP-B* in *TaAFP-BaS* and *TaAFP-BbS* transgenic wheat seeds were detecte d in treated by ABA, NaCl and Mannitol, respectively, for 4d; 3) the trend of transcript expression level of *TaABI5* was same as *TaAFP-Ba* and *TaAFP-Bb* in seeds of transgenic wheat treated by ABA, Salt, NaCl and dehydration, resp

ectively (Fig. 5).

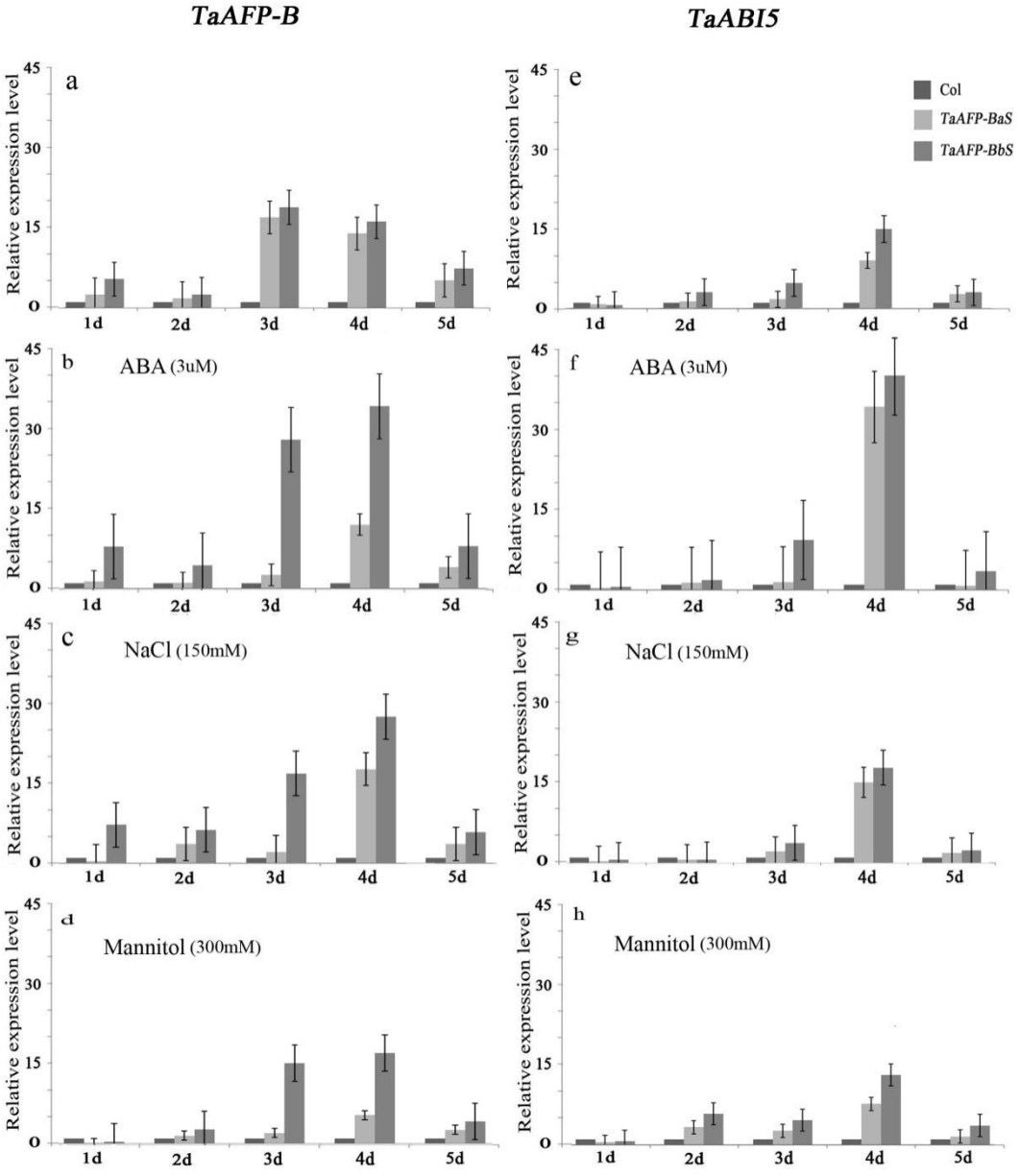

Fig. 5 Expression profiles of *TaAFP-B* and *TaABI5* by qRT-PCR in different stress for 1, 2, 3, 4 and 5d, respectively, in transgenic wheat mature seeds

The observation and statistics of Phenotype in transgenic wheat

The phenotypes (such as plant height, tillering, stem length and the germination index) of single copy positive plant (T2) were observed and analysed (Fig. 6). In transgenic wheat,the average germination index (GI) values of *TaAFP-BaS* and that of *TaAFP-BbS* were 59.79% and 42.58% respectively, indicating a significant difference (P<0.05) compared with control group (55.44%). Compared with the control group, the plant height of *TaAFP-BaS* and *TaAFP-BbS* were 58.91cm and 69.09cm (P=0.0089), and the grain weight of hundred were 3.01kg and 3.59kg (P=0.0002), the rough of second and third stem node of *TaAFP-BaS* were significantly difference (P<0.01). The analysis of variance indicated that most of the phenotype had significant differences (P=0.05) between *TaAFP-BaS* and *TaAFP-BbS* (Fig. 6). These results showed that the 4-bp insertion in the 5'UTR of *TaAFP-B* had an affection on the transcription and translation, and expecially affected the phenotype of GI values, obviously, in overexpression of transgenic wheat system.

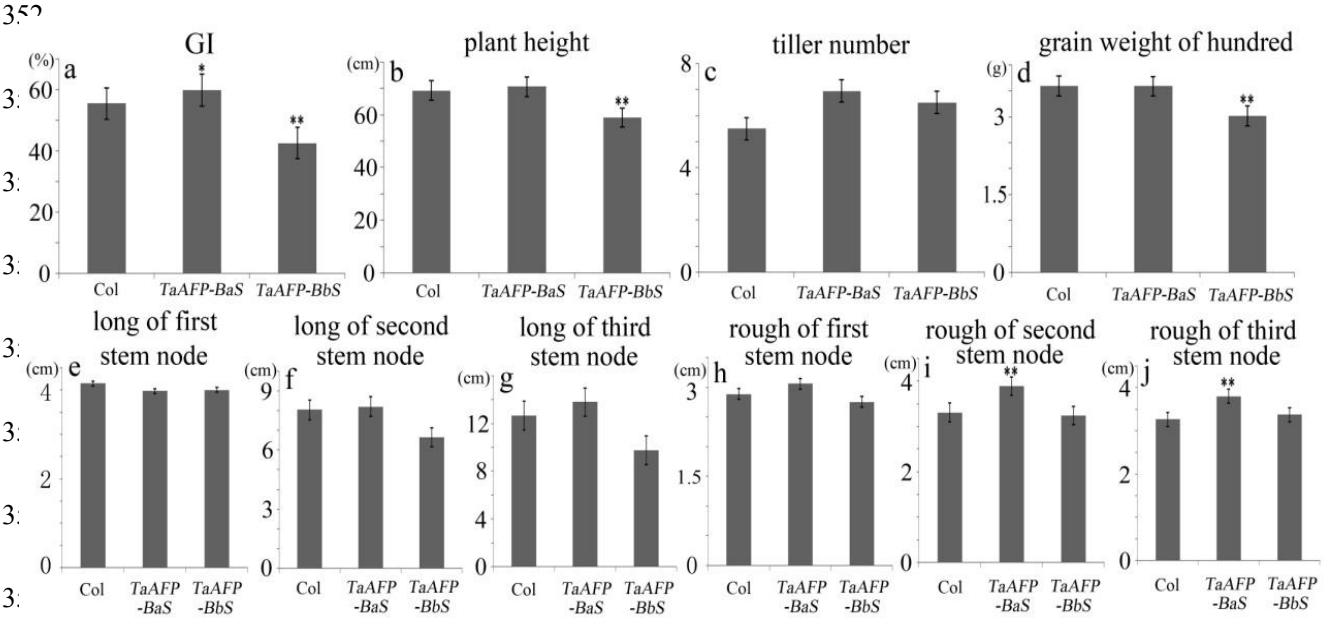

Fig. 6 Analysis and statistic of phenotype in transgenic wheat with sigle-copy of *TaAFP-Ba/bS*

DISCUSSION

The previous study showed that the transcript expression levels of *TaAFP-B* in common wheat with haplotype of *TaAFP-Ba* were markedly higher than those of *TaAFP-Bb* in different tissues , and an interesting experiment date was that the GUS activity of P*TaAFP-Ba*::GUS transgenic rice was more than P*TaAFP-Bb*::GUS in different tissues (Feng et al. 2019). However, different results of transcript expression levels of *TaAFP-B* in wheat lines had been showed that 1) in transgenic wheat, the transcript expression levels of *TaAFP-B* in *TaAFP-BaS* was significantly lower than in *TaAFP-BbS* (Fig 3), in different tissues; 2)but, the average GI values in *TaAFP-BaS* lines of transgenic wheat were higher than in *TaAFP-BbS*. These above two results showed that the more transcript expression level of *TaAFP-B* in transgenic wheat of *TaAFP-Ba/bS* lead to the lower GI values.This result may due to that different receptor with different genetic background effect the phenotypes of transgenic lines, this also were founded by others that single-copy genes had epigenetic silencing in newly synthesized allopolyploids (Comai et al. 2000; Qin et al. 2003). Therefore, the results of this study may be due to the over expression of a single-copy gene of *TaAFP-B* closely related to germination resistance in allohexaploid wheat, leading to the epigenetic silencing. So, experiments of western blotting of *TaAFP-Ba/bS* in transgenic wheat system and functional research of *TaAFP-Ba/bS* in transgenic diploid species need to design for furthermore verification these hypothesises.

During seed maturation and dormancy, ABA also plays an important role during (Gubler et al. 2005; Nakashima et al. 2006), the accumulation and activity of *AFP* were induced by ABA, drought stress, and so on, during seed development and seedling growth, in *Arabidopsis* (Lopez-Molina et al. 2003; Garcia et al. 2008). There had a negative correlation between the germination ability of embryos in different cultivar and the sensitivity of embryos to ABA. Moreover, there is evidence that ABA levels in mature wheat embryos are similar in both PHS-sensitive and -resistant cultivars and that sprouting behaviour is related more to the extent of ABA responsiveness of embryos than to ABA levels (Walker-Simmons et al.1990). In this study, the transcript expression of *TaAFP-BbS* of transgenic wheat seeds treated by

ABA, which of more sensitive than one of *TaAFP-BaS* (Fig.5) Furthermore, there have more higher average GI values in seeds of *TaAFP-BaS* of transgenic wheat than in one of *TaAFP-BbS* (Fig.6). This result showed that 4-bp insertion in 5'UTR of *TaAFP-B* is not only affected the mRNA transcription level, mRNA decay, translation levels of GUS and tdTomatoER and GUS activity (Feng et al .2019), but also ABA sensitive and phenotype of GI value in transgenic wheat. So, allelic variation of *TaAFP-B* (4-bp insertion in 5'UTR) is markedly associated with seed dormancy and plays a role of increasing the GI values and decreasing the pre-harvest sprouting resistance in wheat.

The stable expression of exogenous genes is closely related to the insertion site, copy number, integration efficiency and method of gene transformation in transgenic technology, it is generally believed that multi-copy insertion will affect the stable inheritance and expression of exogenous genes (Zhang et al .2010). So, the copy number of exogenous genes inserted is identified in this study. And the result showed that all of lines were single copy number lines (Fig.2, 3, 4, 5 and 6), which indicating that the agrobacterium-mediated method in wheat transgenic technology lead to more ratio of sigle copy number of exogenous genes inserted of transgenic progeny.

Footnotes:

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Author contributions

YF and YH performed the experiments and wrote the manuscript. BH assisted in performing experiments. YY and YX designed the experiments and assisted in writing the manuscript. All of the authors have read and approved the final manuscript.

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Not applicable.

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Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files. Sequence data used in this manuscript can be found in datebase of NCBI (https://www.ncbi.nlm.nih.gov/) under the following accession numbers:

TaAFP-A(AB360911), *TaAFP-B*(AB360912), *TaAFP-D*(AB360913).

Ethics approval and consent to participate

We declare that these experiments comply with the ethical standards in China where they were performed.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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